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Joint Research Compliance Office

Study protocol: TRUFFLE 2

Perinatal and 2 year neurodevelopmental outcome in late preterm fetal compromise: the TRUFFLE 2 Randomised Trial

Version 4.8

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This protocol describes the TRUFFLE 2 RCT and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Framework for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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GLOSSARY OF ABBREVIATIONS

AE	Adverse Events
APC	Article Processing Charge
cCTG	Computerised Cardiotocography
CI	Chief Investigator
CPAP	Continuous Positive Airway Pressure
CRF	Case Report Form
CTR	Centre for Trials Research
EDC	Electronic Data Capture
FGR	Fetal Growth Restriction
GHQ	General Health Questionnaire
HRA	Health Regulatory Authority
IDMC	Independent Data Monitoring Committee
ISRCTN	International Standard Randomised Controlled Trials Number
ISSHP	International Society for Study of Hypertension in Pregnancy
IVH	Intraventricular Haemorrhage
HIE	Hypoxic Ischemic Encephalopathy
MCA	Middle Cerebral Artery
NHS	National Health Service
NIHR	National Institute for Health Research
NIPPV	Non-Invasive Positive Pressure Ventilation
NNU	Neonatal Unit
PARCA-R	Parent Report of Children's Abilities - Revised for preterm infants
PI	Principal Investigator
PIS	Patient Information Sheet
PPROM	Preterm Prelabour Rupture of Membranes
REC	Research Ethics Committee
ROP	Retinopathy of Prematurity
SAE	Serious Adverse Events
SD	Standard Deviation
SGA	Small for Gestational Age
STV	Short Term Variability
TCTR	The Centre for Trials Research
TMG	Trial Management Group
TRUFFLE	TRial of Umbilical and Fetal FLow in Europe
TSC	Trial Steering Committee
UCR	Umbilical Cerebral Ratio
VEGF	Vascular Endothelial Growth Factor

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KEYWORDS

Fetal Growth Restriction
Ultrasound
Doppler
Stillbirth
Infant neurodevelopment

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AMENDMENT HISTORY

Amendments made post ethics submission.

Amendment No.	Protocol version no.	Date	Summary of changes made since previous version
Substantial amendment 01	4.6	17-Sep-2020	- *REC and ISRCTN reference added* - **UK PIs and Co-investigator:** *PIs updated and new sites added* - **Study Summary:** *Sample size added to Study Summary* - **Trial Management Group:** *additional members added* - ***Data Monitoring Committee:*** *DMC members added* - **Throughout protocol:** *Trial email address updated* - **3. Study Design:** *Update to Umbilical Doppler Delivery Threshold after discussions between clinical TMG members* - **3.1 Study Timeline***: recruitment targets have been updated based on predicted reduced post COVID-19 recruitment rate* - **3.2 Study Outcomes*:*** - *Additions made to ensure the outcomes match the grant application and feasibility study* - *Definition of an infant seizure expanded* - **3.2 Study Outcomes** *Secondary Outcomes expanded to explain the outcomes from the PARCA-R and general health questionnaires* - **4.2 Eligibility Criteria*:*** *Presence of reverse end diastolic flow added to exclusion criteria* - **4.4 Withdrawal*:*** *Withdrawal section expanded to explain the different types of withdrawal* - **5. Safety Reporting*:*** *Adverse events are not being reported for this study. All mention of adverse event reporting has been removed* - **5 Safety Reporting*:*** *SAEs are to be reported on Castor within 24 hours not 48 hours* - **5. Safety Reporting:** *Additional information added to explain the process of the PI assessing seriousness, Causality, and expectedness and the independent clinical review by the CI* - **5. Safety Reporting:** *Serious adverse events will be reported through the trial database and not on paper forms.* - **6. Assessment and follow up:** *Additional text explaining when the follow up questionnaires: general health questionnaire and PARCA-R will be completed* - ***7. Statistical and Data Analysis:*** *Sample size for secondary outcome updated after new PARCA-R analysis* - ***7. Statistical and Data Analysis:*** *main findings analysis updated* - **8. Regulatory***: REC committee added*

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			- **Appendix 2:** *Flowchart updated to change randomisation wording* - **Appendix:** *Inclusion questionnaire has been added*
Substantial Amendment 02	4.7	4-May-2021	- *PI changed for Nottingham University Hospital, new non-UK sites added.* - **Page 5:** *Blood sample collection option added for sub study.* - **Appendix 4:** *Doppler Quality Control Scoring Sheet has been amended to remove the need for the colour Doppler images as these are contained within the composite image supplied by sites.* - **Appendix 4:** *addition of fetal breathing to the scoring for image quality* - **Appendix 5:** *appendix added to describe the optional Biomarker sub study.* - **Appendix 6:** *appendix added to describe the health economics sub study*
Substantial Amendment 03	4.8	TBC	- 2 UK sites added - PI change for Sana Klinikum site - IDMC committee amended. Independent statistician updated. - **3. Study Design** The time frame for repeating the Doppler measurement after an abnormal Doppler has been amended from 2-24 hrs to 15 minutes – 24 hrs. - **3. Study Design** "appropriate clinical reason" added to the reasons why participants could be delivered outside the randomised arm instructions. - **4.2 Inclusion Criteria** The time frame for repeating the Doppler measurement after an abnormal Doppler has been amended from 2-24 hrs to 15 minutes – 24 hrs. - **Appendix 5 Biomarker sub study** *details of study timings and sample collection included* - **Appendix 7 Cardiovascular sub study** *appendix added to describe the optional cardiovascular sub study.*

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PROTOCOL DEVELOPMENT

Protocol Version	Date Created	NIHR Comments and Responses	Collaborator Meetings	Key Changes
Research Plan Version 1	17th January 2019	Research plan submitted to NIHR 17th January 2019	Turin, Italy 29th September 2018 ***47 attendees***	- Observational arm management - Delivery criteria - Doppler measurements 2 abnormal within 24 hours - UCR Cuts off 1.5/1.0/0.5 - Obtain email address at inclusion decision - Centres to use local charts
Research Plan Version 2	20th February 2019	Response to NIHR reviewer comments 20th February 2019	Leuven, Belgium 2nd March 2019 ***51 attendees***	- UCR Cut offs changed to 1.5/1.0 following vote - Timeline
Research Plan Version 3	28th May 2019	Response to NIHR committee 28th May 2019		- Differentiate primary and secondary outcomes - Recruitment review - Two stage consent process
Research Plan Version 4	1st August 2019		London, England 10th July 2019 ***23 attendees in person, 28 via webstream***	- Final protocol discussions - Doppler Standardisation

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STUDY SUMMARY

TITLE	Perinatal and 2 year neurodevelopmental outcome in late preterm fetal compromise: the TRUFFLE 2 randomised, controlled trial.
DESIGN	An individual, two arm, open-label, multicentre, randomised, controlled trial.
AIMS	To establish the optimum method of timing delivery in late-preterm fetal growth restriction.
OUTCOME MEASURES	***The primary outcome*** is poor condition at birth and/or fetal or neonatal death or major morbidity, ***secondary outcome*** two year infant general health and neurodevelopmental outcome based on PARCA-R questionnaires.
POPULATION	Women with non-anomalous singleton ongoing pregnancies 32+0 to 36+6 weeks in whom the estimated fetal weight or abdominal circumference is <10th percentile or has decreased by 50 percentiles since an ultrasound scan at 18-32 weeks.
ELIGIBILITY	As for population, ≥18 years old but with cerebral redistribution as defined by umbilical: cerebral ratio in relation to week of gestation as defined in **appendix 1,** and normal STV on cCTG (≥ 4.5msec).
DURATION	60 months
SAMPLE SIZE	1560 participants

REFERENCE DIAGRAM

Study Flowchart (**appendix 2**)

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1. INTRODUCTION

1.1 BACKGROUND

Third trimester poor fetal growth and physiological compromise are strongly associated with stillbirth, neonatal illness (Baschat, 2018) and a 15% risk of hypoxic brain injury (Miller et al, 2016). The only therapeutic option is delivery of the fetus. This poses a dilemma: delivery too early risks the baby suffering the effects of prematurity, whereas delivery too late risks further fetal compromise increasing the risk of suboptimal outcomes or late stillbirth.

There is little evidence on which to time a decision to deliver such babies. The problem is twofold: there is no consensus on how to identify fetal compromise and an 'ideal' evidence based monitoring strategy remains to be defined. Current screening strategies include standardised symphysis-fundal height measurement, 3rd trimester ultrasound and umbilical Doppler velocimetry (NICE CG62, 2008; RCOG GTG31, 2013). NICE acknowledges that methods to identify antenatal growth restriction are 'poorly developed or not tested by rigorous methodology'. The RCOG recommends that research is required to evaluate the effectiveness of 3rd trimester ultrasound assessment (RCOG GTG 55, 2010). It suggests that 'middle cerebral artery Doppler may be a more useful test in small for gestational age fetuses detected after 32 weeks' (RCOG GTG 31, 2013) but does not define the parameters that should trigger a decision to deliver. Hence, even if there were effective screening for and identification of compromised fetuses, the question would remain as to how to monitor and when to deliver.

A Cochrane Review of the management of 'compromised babies' at term showed no difference in perinatal or long-term outcome with a policy of early delivery versus conservative management (Bond et al, 2015). Only three trials were included: two included small babies, both part of the DIGITAT study (a small pilot and the main trial: Boers et al, 2010). The third included babies with reduced amniotic fluid. There is no Cochrane Review on the optimal time of delivery in late preterm babies.

A systematic review showed that there has only been one trial of late preterm timed delivery. The Growth Restriction Intervention Trial (GRIT Study Group 2003, 2004) included 210 babies at risk of late preterm growth restriction or compromise between 33+0 and 36+6 weeks. Of the late preterm babies recruited to the GRIT study, 107 were randomised to early delivery and 103 to delayed delivery. Mortality and a range of neurodevelopmental measures were similarly distributed between the groups. These results cannot be used to inform management because of the small number of infants were assessed using only one Doppler measure (umbilical artery Doppler) and visual inspection of the cardiotocograph (not computerised analysis), and management prior to delivery was not standardised, but left to the clinician's discretion.

Doppler ultrasound of the fetus allows non-invasive and detailed assessment of the impedance to blood flow in fetal vessels. Ultrasound markers of compromise include cerebral blood flow redistribution (Meher et al, 2015; Khalil et al, 2016; Akolekar et al, 2015) and abdominal circumference growth velocity 'drop off' on the 3rd trimester (Sovio et al, 2015; Gordijn et al, 2016). Fetal 'cerebral redistribution', prioritising blood flow toward the brain is a fetal response to a hostile intrauterine environment characterised by falling oxygen levels (hypoxia). Intervention by delivery of the fetus in response to either cerebral Doppler or fetal growth slowing has never been tested in a randomised trial. Computerised cardiotocography (cCTG) has been used in several observational studies.

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We have previously carried out a randomised trial of delivery decision-making using Doppler velocimetry and cCTG in more premature fetuses with growth restriction – the Trial of Randomised Umbilical Fetal FLow in Europe (TRUFFLE) 1 which provided evidence associating monitoring strategies with outcomes (Lees et al, 2015) and guides practice internationally. In the TRUFFLE 1 study we studied early onset preterm growth restriction using cCTG, as part of a RCT comprising three strategies, including fetal Ductus Venosus Doppler. This was associated with reduced neurodevelopment in survivors at 2 years of age (Lees et al, 2015). However, blood flow changes over the last trimester-and in particular cerebral Doppler, have not been systematically evaluated against fetal outcomes. In the TRUFFLE 1 study, no women were entered in the study after 32 weeks.

From the TRUFFLE 2 Feasibility Study (2017-18, n=1024) (Stampalija et al, 2020), we have evaluated cerebral redistribution index delivery trigger points in two gestational age bands. Clinician feedback at TRUFFLE meetings (Berlin 2017 & Turin 2018) suggests that greater concern about fetal condition is required to trigger delivery at earlier gestational ages compared, for example, to delivery at 36 weeks of gestation. Hence, we have selected a more abnormal 'cut off' for cerebral redistribution index at earlier gestational ages, based on a graduated range of umbilical cerebral ratio z scores as described below. This was considered important to avoid iatrogenic prematurity. Of note from these data is the finding (never previously reported in a prospective cohort) that the birth asphyxia, fetal mortality and neonatal morbidity rate is higher (15%) for fetuses showing cerebral redistribution (ie: those with a higher UCR z score) than those with a lower UCR z score (9%).

This randomised trial of delivery in late fetal growth restriction and compromise using Doppler velocimetry and computerised cardiotocography (TRUFFLE 2) is designed to investigate the optimal timing of delivery in late onset fetal growth restriction.

1.2 RATIONALE FOR CURRENT STUDY

There is no consensus on which fetal monitoring modalities should trigger delivery in fetal growth restriction between 32 and 36+6 weeks gestation, practice varies widely within the UK and worldwide. The consequences of inappropriately early or late delivery for perinatal and infant health and maternal morbidity are potentially enormous. Current interest lies in the use of cerebral Doppler indices in the timing of delivery to optimize fetal outcomes, though little evidence from prospective studies exists.

We hypothesise that delivery on the basis of cerebral blood flow redistribution reduces a composite of perinatal poor outcome, death and short-term hypoxia-related morbidity.

2. STUDY OBJECTIVES

The aim of this project is to determine, in situations where fetal growth is poor or fetal size is small between 32 and 36+6 weeks of gestation, whether delivery on the basis of cerebral blood flow redistribution reduces adverse outcome compared with waiting until the fetal heartrate pattern suggests possible hypoxaemia/acidosis on cCTG.

Our objective is to carry out a randomised controlled trial to test the hypothesis that delivery on the basis of cerebral blood flow redistribution reduces a composite of perinatal poor outcome, death and short-term hypoxia-related morbidity (efficacy outcome). Secondary safety outcomes, include follow-up of babies to assess neurodevelopmental outcome at two years.

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3. STUDY DESIGN

Multicentre individually randomised controlled trial involving 11 UK as well as European & International centres to identify the optimum method to time delivery of late preterm growth restricted fetuses or at risk of compromise in order to improve perinatal and long-term outcome.

Potential participants are women with singleton non-anomalous ongoing pregnancies 32+0 to 36+6 weeks in whom a small for gestational age (SGA) baby is identified or one whose growth has slowed. This is defined as estimated fetal weight or abdominal circumference <10th percentile or decreased by 50 percentiles since an ultrasound scan at 18-32 weeks. Each centre will utilise their local growth charts. Data will be collected on absolute measurements as well as the growth chart used to calculate centiles. Once identified as potential participants they will receive regular monitoring as per the usual standard of care of fetal condition using ultrasound of biometry, cerebral Doppler assessments and cCTG (using Dawes-Redman criteria). This is recommended to be every 14 days. This observational data will be recorded.

Women become eligible for randomisation once signs of compromise are detected by Doppler cerebral redistribution, as defined below. The sample size is 1560 randomised patients.

We have selected the UCR pulsatility index z-scores, depending on gestation, of 1.5 (32+0 - 33+6 weeks) and 1.0 (34+0 - 36+6 weeks). These correspond to a UCR of ≥1.0 at 32+0 to 33+6 weeks and ≥0.8 at 34+0 to 36+6 weeks. Abnormal UCR measurements must be repeated within 15 minutes – 24 hours to confirm compromise. If the second abnormal Doppler is collected within 36 hours of the first abnormal Doppler, the participant will not be deemed ineligible and randomisation will still be possible. The second abnormal Doppler (qualifying Doppler for randomisation) must be completed by a sonographer who has been signed off on the site staff delegation log and has been reviewed by the local PI (see section 3.3). Randomisation will occur at the time of the second qualifying UCR measurement and will be stratified by centre and gestational age. The two abnormal Doppler results need not be consecutive. If the second Doppler measurements are normal following an initial abnormal measurement, the patient may be randomised at the time of the next abnormal Doppler as long as it is within 72 hours of the first abnormal result. Randomisation will be through the CASTOR website (Amsterdam, NL) into which eligibility, monitoring and outcome data are entered.

Women will be consented in a 2 stage process. Stage 1 (pre-eligible) consent is not a prerequisite and women may be consented directly for randomisation with stage 2 (eligible for randomisation) consent.

Pre-eligible

1. Consented for prospective data collection once identified as meeting SGA criteria or slowed fetal growth (as defined above) but not meeting Doppler thresholds for randomisation. This will include demographics, medical history, ultrasound findings and outcomes. This consent will include obtaining contact details which will be entered into and stored on Castor and optional permission for blood samples to be taken for the biomarker sub-study, **see Appendix 5.** Participants will be sent an inclusion survey via email to collect socio-economic and demographic information.

Eligible for randomisation

2. Consent for randomisation once cerebral redistribution is identified, with UCR (as defined above) of ≥1.0 at 32+0 to 33+6 weeks and ≥0.8 at 34+0 to 36+6 weeks. This

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consent will also include a personal email address and willingness to be contacted in the future for follow up.

Women will be randomised to either immediate delivery or delayed delivery as defined below.

Immediate Delivery
Participants in the immediate delivery arm will be delivered or induction of labour commenced within 48 hours, allowing for administration of corticosteroids and infusion of magnesium sulphate as per local protocol and guidance (appendix 3). Start of induction of labour is defined as administering cervical preparation (balloon, prostaglandins, etc) or artificial rupture of membranes or administration of oxytocin.

Delayed Delivery
Participants in the delayed delivery arm will be closely monitored using at least twice weekly Doppler and cCTG monitoring, or more frequently based on local centre protocols for clinical management. Umbilical Doppler may be measured in this time and delivery may be based on these safety net criteria (see box below). We strongly recommend that MCA measurements are not undertaken in the delayed delivery arm. Delivery is indicated when STV <4.5msec on cCTG or there are repeated decelerations. Once participants reach 37^{+0} weeks gestation the delivery plan should revert to local protocol.

Delivery in either arm, outside of the process described above, can be based on the Umbilical Doppler delivery thresholds below or a clinical reason deemed appropriate by the medical team.

Umbilical Doppler delivery thresholds

In all arms absolute indications for delivery include:
- umbilical artery Doppler with reversed end diastolic flow after entry into the trial, OR
- umbilical artery Doppler absent end diastolic flow from 34^{+0} weeks

3.1 STUDY TIMELINE

Study set up: 0-6 months; recruitment/randomisation: 7-30 months, with recruitment review* decision-point at 18 months. Two year follow up questionnaires 31-54 months; analyses, writing up, reporting & dissemination: 55-60 months. This equates to 5 years (60 months).

*Recruitment review will be undertaken based on 9 months recruitment following the set up phase (i.e. 15 months from the start date of study) assessed at one year after recruitment starts (i.e. 18 months from the start date of study). By this point the stop/go criteria will be as follows:

1. Recruitment of 257 participants.
2. That by this point 60% of the centres (21 centres) will have been open to recruitment for at least six months.
3. That of these 21 centres with six months recruitment experience, at least 15 will have recruited seven or more participants.

3.2 STUDY OUTCOME MEASURES

Outcome measures: Primary

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Composite poor condition at birth and neonatal adverse outcome
Any of the following:
1) Poor condition at birth
   - Apgar score at 5 minutes <7, Arterial pH of <7.0 or venous pH of <7.1
   - Resuscitation with intubation, chest compressions or medication

2) Fetal death/ Death before neonatal hospital discharge

3) Neonatal brain injury syndromes
   - Infants with a diagnosis consistent with hypoxic ischaemic encephalopathy: term and near-term infants only
   - Infants with a diagnosis of intracranial haemorrhage, perinatal stroke, hypoxic ischaemic encephalopathy (HIE), central nervous system infection, and kernicterus (bilirubin encephalopathy): all infants
   - Preterm white matter disease (periventricular leukomalacia): preterm infants only
   - Infants with a recorded seizure confirmed by EEG

4) Respiratory support
   - Need for mechanical support of respiration after admission to NNU, for more than 1 hour – includes need for continuous positive airways pressure (CPAP or NIPPV) or mechanical ventilation via an endotracheal tube – but excludes need for supplemental oxygen.

5) Cardio-vascular abnormality
   - Hypotensive treatment, ductus arteriosus treatment, or disseminated coagulopathy;

6) Sepsis (clinical sepsis with positive blood culture, or necrotising enterocolitis requiring surgery)

7) Retinopathy of prematurity requiring treatment (laser or anti-VEGF injections)

Outcome measures: Secondary *For the baby:*
Health and developmental outcomes assessed using – PARCA-R questionnaire at 2 years corrected age and the general health questionnaire up to 2 years.*

The PARCA-R (Parent Report of Children's Abilities-Revised) (Johnson et al, 2019) will be completed at 24 months from correct age. It allows the following scales to be derived: Non-verbal cognitive scale and language development scale. Raw scores from the scales are standardised (by corrected age and gender) to a notional population mean of 100 SD=15 and the average of these two component scores will be taken as the Overall composite score. Corrected age is where preterm babies (born before 37 weeks) use estimate date of delivery as opposed of date of birth.

The general health questionnaire (GHQ) (Chappell et al, 2019) will be used to derive the following health outcomes at 6, 12, 18 and 24 months post-partum:
- Use of ANY hospital service (yes/no) and total number of contacts over the 2 year period;
- Admitted to hospital (yes/no) and total number of admissions over the 2 year period;
  - Planned/unplanned admissions to hospital (yes/no) over the 2 year period;
  - Intensive care or not over the 2 year period;
- Attended A&E (and not subsequently admitted) (yes/no) over the 2 year period;
- Attended Outpatients/clinic (yes/no) over the 2 year period.

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For the mother:

1) Gestational hypertension
   - As defined by the International Society for Study of Hypertension in pregnancy (ISSHP): hypertension (blood pressure ≥140/90mmHg) arising de novo after 20 weeks gestation in the absence of proteinuria

2) Pre-eclampsia
   - As defined by the ISSHP: (blood pressure ≥140/90mmHg AND significant proteinuria (protein/creatinine ratio of 30 mg/mmol)

3) Onset of labour (spontaneous, induction (method), prelabour cesarean)

4) Mode of delivery (spontaneous vaginal, assisted vaginal, caesarean)

*Researchers will send the general health and PARCA-R questionnaires via encrypted email address up to 24 months corrected age to be completed by the parents. This longer-term follow up is only for eligible participants who were randomised (and is not required for pre-eligible patients who were never randomised). The parents will complete the web-based forms via a hyperlink sent to their email address. After the questionnaires are undertaken by the parents, they will be linked back to their pseudo-anonymised records on the CASTOR website. It is the responsibility of the PI from each centre to ensure that these are sent out, however this can be done by anyone within the centre's research team.

A health economic analysis will be conducted using an anonymised version of the study dataset, **see Appendix 6.**

3.3 DOPPLER QUALITY CONTROL

Sonographer Standardisation: Each sonographer in each centre taking part in the study will be standardised by the local principal investigator (PI). Each sonographer will submit to the local PI a total of four images: two pseudo anonymised ultrasound images for each Doppler parameter (umbilical artery and MCA) each showing a colour Doppler image with the gate placed over the vessel, and separately an image showing the pulsed wave Doppler waveform arising from that image. The local PI will determine whether these images are satisfactory using a predefined quality control scoring system (see appendix 4).

Doppler Ultrasound Criteria: Measurements will be obtained in fetuses between 32+0 and 36+6 weeks of gestation. Doppler assessment of the umbilical artery and MCA pulsatility index images will be collected according to specific predefined objective criteria for both the colour Doppler images and pulsed wave Doppler.

Doppler Quality Control: The local PI will provide details of all sonographers having undergone standardisation in that centre to the Centre for Trials Research. The Centre for Trials Research will independently request all images submitted to the local PI for the first 5 patients randomised from each unit, and then up to 10% of patients thereafter, for anonymised quality control assessment by the Quality Control Board. All images will be collected as pseudo anonymised .jpeg images and saved electronically in a Doppler ultrasound sonographer standardisation file by the PI, with the submitting sonographer identifiable. Images will be scored using the predefined scoring criteria. The CTR will manage this process and will provide feedback if necessary, and will ensure that members of the TRUFFLE 2 Quality Control Board do not assess images from their own unit.

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4. PARTICIPANT ENTRY

4.1 PRE-REGISTRATION EVALUATIONS

- Ultrasound scan of fetal growth between 32+0 and 36+6 weeks including measurement of middle cerebral artery Doppler and umbilical artery Doppler
- cCTG analysed using Dawes-Redman criteria

4.2 INCLUSION CRITERIA

(All criteria should be fulfilled to be eligible for randomisation)

- Women ≥ 18 years old
- Pregnant with singleton non-anomalous fetuses
- Between 32+0 and 36+6 weeks of gestation
- Estimated fetal weight or abdominal circumference <10th percentile OR decreased by 50 percentiles since an ultrasound scan at 18+0-32+0 weeks and:
- Cerebral redistribution defined as UCR ≥1.0 (32+0-33+6 weeks) or ≥0.8 (34+0-36+6 weeks) **repeated within 15 minutes to 24 hours**
- Normal STV on cCTG (4.5msec or above)

4.3 EXCLUSION CRITERIA

- Indication for immediate delivery required within 48 hours
- Unable to give informed consent
- Preterm prelabour rupture of the membranes (PPROM)
- Suspected placental abruption or antepartum haemorrhage
- Presence of reversed end diastolic flow in the Umbilical Artery

4.4 WITHDRAWAL

Clear contact details for investigators at each unit are clearly presented on the Patient Information Sheet (PIS), and communication between participants and investigators is encouraged throughout the study. Participants should inform any member of the study team of their wish to withdraw consent. All data collected up to the point of withdrawal will be included in the analysis.

There are two types of withdrawal:

1. Participants may withdraw consent to follow the allocated treatment arm at any time. Such requests will be respected, and their delivery timing decision recorded. Such participants will be followed up and their outcome data analysed by “intention to treat”.
2. Participants may also withdraw consent to continued data collection. Such decisions will also be respected, but data already collected will be used. We will inform such participants that withdrawing from further data collection risks the integrity of the trial. If they still do not wish to have any further data collected, this will be recorded on Castor and no further data entry will be possible

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5. SAFETY REPORTING –SERIOUS ADVERSE EVENTS

The Principal Investigator is responsible for ensuring that all site staff involved in this trial are familiar with the content of this section.

All Serious Adverse Events (SAEs) must be reported immediately (and within 24 hours of knowledge of the event) by the PI at the participating site to the CI and Centre for Trials Research (Cardiff University) Trial Team unless the SAE is specified as not requiring immediate reporting (see section 5.2 - excluded SAEs).

5.1 Definitions

Table 1 Serious Adverse Event Definition

Term	Definition
Serious Adverse Event (SAE)	Any adverse event that - • Results in death • Is life-threatening* • Required hospitalisation or prolongation of existing hospitalisation** • Results in persistent or significant disability or incapacity • Consists of a congenital anomaly or birth defect • Other medically important condition***

***Note:** The term 'life-threatening' in the definition of serious refers to an event in which the trial participant was at risk of death at the time of the event or it is suspected that use or continued use of the trial procedure would result in the subjects death; it does not refer to an event which hypothetically might have caused death if it were more severe.

**** Note:** Hospitalisation is defined as an inpatient admission, regardless of the length of stay, even if the hospitalisation is a precautionary measure for continued observation. Pre-planned hospitalisation e.g. for pre-existing conditions which have not worsened, or elective procedures, does not constitute an SAE.

***** Note:** other events that may not result in death, are not life-threatening, or do not require hospitalisation, may be considered as an SAE when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

5.2 Trial Specific SAE Reporting requirements

Due to the specific study population involved in the TRUFFLE 2 trial, some serious adverse events will be expected. Events that are deemed to be due to normal pregnancy and delivery, based on clinical grounds, will be exempt from reporting (see below list for excluded SAEs). Serious adverse events will not be reported for women in the pre-eligible (Stage 1) phase of the study because they will only receive standard medical care. Serious adverse events should be reported from time of signature of informed consent for the randomisation phase, throughout the treatment period up to discharge home for the mother or neonate for all events, except maternal death which will be reported up to 42 days after delivery. Adverse events whether they are expected or not should be recorded in the patient's hospital notes.

Included SAEs

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The SAEs which should be reported are:-

For the mother:-

- Maternal Death from trial inclusion up to 42 days after delivery (WHO, 2016)
- All other events which meet the description of SAEs in section 5.1 which are not in the excluded SAEs below.

For the baby:-

- Fetal death
- Neo-natal death before first discharge home
- All other events which meet the description of SAEs in section 5.1 which are not in the excluded SAEs below.

These should be completed in the participant's notes and on the relevant Castor EDC form, and notified to the CTR in the normal timeframes.

Excluded SAEs

The SAEs expected in this trial but for the purposes of this trial do not need to be reported are:

For the mother:

- Pre-eclampsia / eclampsia
- Gestational hypertension
- Caesarean section
- Assisted vaginal delivery

For the baby:

- Neonatal morbidity (collected as primary outcome)

All of the above will be collected as outcomes on Castor EDC and therefore do not need to be reported as SAEs.

The Principal Investigator (or another delegated medically qualified doctor from the trial team) will assess each SAE to determine the seriousness, causality, and expectedness. The Chief Investigator (or another appropriately qualified co-investigator) will then be approached by the CTR to complete an independent clinical review of the SAE for causality and expectedness.

5.3 Causality

Causal relationship will be assessed for the intervention and procedures:

Procedures:
• Ultrasound scan • cCTG • Induction of labour • Caesarean delivery

Table 2 Causality Definitions

Relationship	**Description**	**Reasonable possibility that the SAE may have been caused by the intervention?**

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Unrelated	There is no evidence of any causal relationship with the intervention	
Unlikely	There is little evidence to suggest there is a causal relationship with the intervention (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).	
Possible	There is some evidence to suggest a causal relationship with the intervention (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).	
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	
Definite	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	

The causality assessment given by the Principal Investigator (or delegate) cannot be downgraded by the Chief Investigator (or delegate), and in the case of disagreement both opinions will be provided.

5.4 Expectedness

The Chief Investigator (or another delegated appropriately qualified individual) will assess each SAE to perform the assessment of expectedness. This assessment will we based on whether the event is a recognised complication of fetal growth restriction or pre-eclampsia, or a recognised result of the management of these conditions.

SAEs which add significant information on specificity or severity of a known, already documented adverse event constitute unexpected events. For example, an event more specific or more severe than that described in the protocol is considered unexpected.

Table 3 Table of Examples of expected events for the intervention and/or procedures

Induction of Labour *Inducing labour NICE Clinical guideline [CG70] Published date: July 2008*	**Caesarean section** *Royal College of Obstetricians and Gynaecologists Consent Advice No. 7 Caesarean Section October 2009*
• Tachysystole / hyperstimulation	• Infection
• Uterine rupture	• Haemorrhage / blood transfusion

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• Failed induction / cesarean section	• Hysterectomy
• Instrumental delivery	• Admission to intensive care
• Regional anaesthesia	• Damage to bladder / bowel / ureters (and repair thereof)
• Perineal trauma	• Return to theatre / readmission to hospital
	• Fetal laceration
	• Venous thromboembolism

5.5 Reporting procedures

5.5.1 Participating Site Responsibilities

The PI (or delegated appropriately qualified doctor from the trial team) should sign and date the SAE form electronically on the Castor database to acknowledge that they have performed the seriousness, causality and expectedness assessments. Investigators should also report SAEs to their own health boards or trust in accordance with local practice.

A completed SAE form for all events requiring immediate reporting should be completed on the Castor Database within 24 hours of knowledge of the event. An email should be sent to TRUFFLE2@cardiff.ac.uk to notify them that an SAE has been reported on Castor.

The participant will be identified only by trial identification number. The participant's name should not be used on any correspondence.

It is also required that sites respond to and clarify any queries raised on any reported SAEs and report any additional information as and when it becomes available through to the resolution of the event. Additionally, the CTR may request additional information relating to any SAEs and the site should provide as much information as is available to them in order to resolve these queries.

Report SAEs on Castor

For any queries please contact CTR: truffle2@cardiff.ac.uk

An SAE form is not considered as complete unless the following details are provided:

- Full participant trial number
- An Adverse Event
- A completed assessment of the seriousness, and causality as performed by the PI (or another appropriately medically qualified doctor registered on the delegation log).

If any of these details are missing, the site will be contacted, and the information must be provided by the site to the CTR within 24 hours.

5.5.2 The CTR responsibilities

Following the initial report, all SAEs should be followed up to resolution wherever possible, and further information may be requested by the CTR. As information becomes available it can be added to the SAE form on Castor.

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Sites should continue reporting SAEs until 42 days after delivery for maternal death, and up to first discharge home for neonatal death.

Once an SAE is received at the CTR, it will be evaluated by staff at the CTR and sent to the Chief Investigator (or their delegate) for an assessment of expectedness.

For all non-CTIMP studies, including clinical investigations of medical devices, only reports of related and unexpected Serious Adverse Events (SAEs) should be submitted to the REC. These should be sent within 15 days of the chief investigator becoming aware of the event. There is no requirement for annual safety reports in addition to the information provided through the annual progress report.

6. ASSESSMENT AND FOLLOW-UP

Assessment of the primary outcome will be at infant discharge from the NNU and assessment of the key secondary infant outcomes using the general health questionnaire and PARCA-R. The general health questionnaire will be sent out by the Castor website at 6, 12, 18 and 24 months post-partum. Neurodevelopment will be assessed at 2 years age corrected for prematurity using parent-report: PARCA-R. The window for determining 2 year outcome will be from 22-28 months over which range the PARCA-R has been standardised.

Endpoint will be 30 months after the estimated date of delivery of the last participant to deliver (24 months follow-up with additional 6 months for data cleaning and additional enquiries).

7. STATISTICS AND DATA ANALYSIS

Sample Size: Primary Outcome
The trial is powered to detect if delivery following cerebral redistribution is superior to expectant management following cerebral redistribution on this outcome. A difference in the proportion with the primary outcome from 15% in the delayed delivery to 9% in the immediate delivery (from TRUFFLE 2 feasibility study) demonstrates an odds ratio of 0.560. At 2-sided 5% significance with 95.5% power, 780 participants per arm are required, giving 1560 in total. Given the immediacy of this outcome, no loss to follow is expected.

Sample Size: Secondary Outcome
An important secondary outcome is neurodevelopment, which will be measured at two years using a parental questionnaire including PARCA-R, a well validated parent report questionnaire to quantify developmental attainment used in large trials in perinatal medicine (www.parca-r.info) and recommended in NICE Guidance (https://www.nice.org.uk/guidance/QS169).

Assuming a loss to follow-up at two years of 20% we should obtain long term outcomes for approximately 1248 infants (624 per group assuming no difference in the loss to follow-up between the groups). The PARCA-R questionnaire provides a composite score for neurodevelopment with a standardised mean of 100 and standard deviation of 15. With a one-sided significance level of 1%, under a non-inferiority hypothesis, a sample size of 624 in each group achieves a 98% power to detect a non-inferiority margin of difference in the mean PARCA-R score of no less than 4 points (0.25 of a standard deviation). A margin of no less than 3 points can be detected with 90% power.

Main analysis
The primary analysis for the primary outcome of composite of adverse fetal/neonatal outcomes will be intention to treat with participants analysed in the groups to which they are assigned

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regardless of deviation from the protocol or intervention received. This outcome is a composite of adverse fetal/neonatal outcomes and comprises:
Poor condition at birth, fetal death/ death before neonatal hospital discharge, neonatal brain injury syndromes, respiratory support, cardio-vascular abnormality, sepsis and retinopathy of prematurity requiring treatment. We consider that the intervention will reduce the frequency of all components of this outcome. The outcomes from the GHQ will be analysed similarly. The PARCA-R will be both an intention to treat and a per protocol analysis, since the hypothesis under examination for these outcomes is a non-inferiority hypothesis. The per-protocol analysis will exclude babies of women who do not receive the allocated intervention as per protocol and will be further defined in the Statistical Analysis Plan.

As the trial includes a reasonable number of centres (and will involve a reasonable number of participants randomised per centre), the analysis will be based on the individual participant, allowing for clustering between participants within centre using robust standard errors. All analyses will additionally adjust for gestational age at inclusion (stratification risk factor used in randomisation) as a fixed factor. For binary outcomes a logistic regression model will be used to compare this outcome by arm and results will be presented as odds ratios and two-sided 95% confidence interval (CI). Continuous outcomes will be analysed using linear regression and results presented as adjusted differences in means alongside 95% CIs.

Additional pre-specified sub-group analyses will be carried out to analyse morbidity in the whole cohort by those that are <10th centile and <3rd centile based on intergrowth, WHO and customised growth charts. Morbidity will also be analysed by maternal morbidity (yes/no), corticosteroid administration (yes/no) and mode of delivery (Spontaneous or Operative vaginal/ Caesarean section).
Both analyses will be undertaken after database lock following data collection at 2 years. No interim analyses are planned. Missing outcome data but will be accounted for in sensitivity analyses using multiple imputation, where we will assume that outcome data are missing at random given the observed measurements.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

A detailed statistical analysis plan will be written prior to analysis. The reporting of findings will be in accordance with the CONSORT guidelines for RCTs. Statistical analysis will be performed in Stata (version 16 or higher).

8. REGULATORY ISSUES

8.1 ETHICS APPROVAL (UK)

The Study Coordination Centre has obtained approval from the London - Riverside Research Ethics Committee (REC) and Health Regulatory Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions. In non UK countries, each centre will apply for local research ethics approval for the study.

8.2 CONSENT

Consent to enter the study will be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent will be obtained. The right of the participant to refuse to participate without giving reasons will be respected. After the participant has entered the study the clinician

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remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so will be recorded. In these cases the participants will remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

For detailed information on consent and withdrawal please see Section 3: Study Designs, and Section 4.4: Withdrawal.

8.3 CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

8.4 INDEMNITY

Imperial College London confirms that negligent harm and non-negligent harm insurance policies which apply to this study are in place.

The following additional Exceptions apply to the Policy:
The Underwriters shall not be liable to indemnify the Insured under this Policy in respect of any liability, claim, loss, costs or expenses arising out of, caused by, resulting from, in consequence of, in connection with or in any way directly or in-directly involving birth defects.

and

The Underwriters shall not be liable to indemnify the Insured under this Policy in respect of any liability, claim, loss, costs or expenses arising out of, caused by, resulting from, in consequence of, in connection with or in any way involving any injury or death to any foetus.
All other terms, conditions, limitations and exclusions of the Policy remain unaltered.

8.5 SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

8.6 FUNDING

NIHR HTA is funding this study.

8.7 AUDITS

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Frame Work for Health and Social Care Research.

9. STUDY MANAGEMENT

The Chief Investigator (CI), Christoph Lees (Imperial College, London), will have responsibility for the conduct of the study. The Sponsor of the trial will be Imperial College, London. Neil Marlow (NM) is responsible for neonatal and infant follow up and Hans Wolf (HW-Amsterdam) for website/data collection. All UK & European co-investigators are listed at the top of this document.

The Centre for Trials Research (CTR), Cardiff University (CU) will be responsible for the co-ordination and the management of the trial, with the trial manager acting as the day-to-day point of contact. CTR, a UKCRC registered trials unit, will manage the trial according to required regulations and governance, through their Standard Operating Procedures, including those for project management and study committee structure, data management (including

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routine data) and protection, adverse/serious adverse event reporting, maintaining study documentation and archiving study data. CTR staff are co-applicants on this proposal and have been integral to the process of study design, methodology, research planning, and sample size calculation. CTR staff will be members of the Trial Management Group (TMG), providing advice on, and oversight of, the project throughout, including randomisation, trial and data management, statistical analysis, reporting, dissemination and archiving. To facilitate, the following governance structures will be instituted:

- Weekly project team meetings which the trial manager will Chair and will be attended by all CTR staff working on the trial, the CI and other appropriate team members dependent on the stage of the trial.
- Trial management group (TMG) meetings, chaired by the CI, and attended monthly by co-investigators and members of the project team meeting to discuss the trial progression and any management issues. All UK and European investigators will meet 6 monthly in either UK or European venues.
- Trial steering committee (TSC) to provide independent supervision, safety assessment and oversight of the trial. They will meet initially to review the protocol, and thereafter, at least annually, to advise on the conduct and progress of the study, and any relevant practice and policy issues.
- Independent Data Monitoring Committee (IDMC) will meet at least annually to monitor accumulating data on safety and any trial intervention benefit.

TMG, TSC, and IDMC members will be required to sign up to the remit and conditions set out in separate Charters for each group. These Charters will explain clearly the inter-relationships and responsibilities of the different groups. The project will employ standardised research protocols, which will be agreed and monitored by the TMG, TSC and IDMC. The trial will be registered, prior to recruitment, for an International Standard Randomised Controlled Trials Number (ISRCTN).

The clinical trial risk assessment has been used by Centre of Trial Research to determine the intensity and focus of central and on-site monitoring activity in the TRUFFLE 2 trial. Appropriate monitoring levels will be employed and are fully documented in the trial monitoring plan.

Investigators should agree to allow trial related monitoring, including audits and regulatory inspections, by providing direct access to source data/documents as required. Participant consent for this will be obtained.

Findings generated from on-site and central monitoring will be shared with the Sponsor, CI, PI & local R&D.

10. PUBLICATION POLICY

This will be in line with NIHR Open Access policy. NIHR Open Access policy relates to any peer-reviewed research supported in whole or in part by the NIHR. The NIHR require that funded researchers seek to publish outputs in a peer reviewed journal that is compliant with the NIHR's Open Access policy which states that any Article Processing Charge (APC) paid for by the NIHR must ensure that the output is made available using the Creative Commons Attribution (CC BY) licence, and allows immediate deposit of the final published version in other repositories without restriction on re-use.

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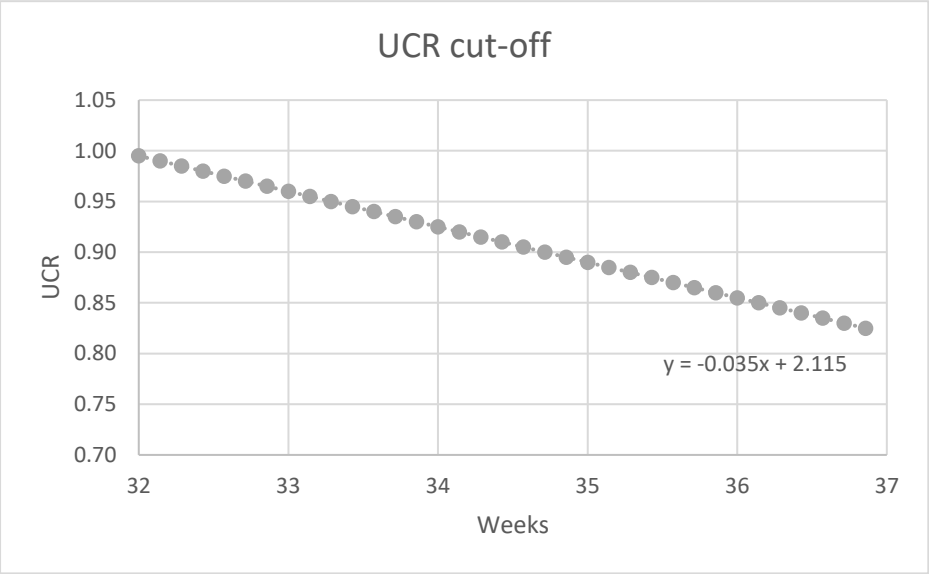
APPENDIX 1: UCR CUT-OFF VALUES

The values follow a line from (approximately) UCR Z-score 1.5 at 32 weeks to UCR Z-score 1.0 at 37 weeks. Function: UCR = -0.035 x [Gestational age (weeks)] + 2.115. For randomisation UCR should be larger than the cut-off value for GA at measurement.

weeks	Cut-off UCR gradual	Cut-off UCR 2-step	Cut-off UCR 2-step simple
32	1.00	0.98	1
33	0.96	0.98	1
34	0.92	0.83	0.8
35	0.88	0.83	0.8
36	0.84	0.82	0.8



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APPENDIX 2: TRUFFLE 2 FLOWCHART

TRUFFLE 2 flowchart

32+0 to 36+6 weeks, singleton, non-anomalous pregnancy, age ≥ 18

AND

EFW or AC <10th centile ***OR*** decrease by 50 percentiles

AND

Normal computerised CTG (STV ≥ 4.5msec)

AND

Umbilical Cerebral Ratio ≥ 1.0 at 32+0 - 33+6 weeks
≥ 0.8 at 34+0 - 36+6 weeks

(UCR must be repeated within 15 minutes – 24 hours to confirm abnormal reading)

AND

No contra-indication to either trial arm

RANDOMISATION (at time of 2nd abnormal UCR)

1:1 central by centre and gestational age (above or below 34 weeks)

IMMEDIATE DELIVERY

Delivery or start of induction of labour within 48 hours of randomisation

To allow for administration of corticosteroids and magnesium sulphate (see guidance)

DELAYED DELIVERY

Twice weekly ultrasound Doppler and cCTG

Delivery mandatory if: cCTG STV < 4.5 msec

OR repeated decelerations

OR if UA EDF is reversed

OR ≥ 34 + 0 if UA EDF absent

PRIMARY OUTCOME – Composite perinatal outcome

SECONDARY OUTCOMES – 2 year neonatal follow-up & maternal outcomes

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APPENDIX 3: CLINICAL GUIDANCE

The following consensus guidance is offered in relation to management of women entered into the study where specific items of management are not mandated by the protocol.

- We recommend maternal corticosteroid administration for fetal lung maturation up to 34^{+0} weeks.
- Consider corticosteroids for elective Caesarean deliveries according to local protocols.
- We recommend magnesium sulphate infusion for neuroprotection up to 34^{+0} weeks.
- Women with SGA babies and normal Doppler readings (pre-eligible) should have biometry and Doppler assessment at least every two weeks.
- Women in the delayed delivery arm should not have MCA measurements as part of monitoring.
- Beyond 37^{+0} weeks, delivery decisions and management are as per local protocol.

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APPENDIX 4: DOPPLER QUALITY CONTROL SCORING SYSTEM

For Umbilical Artery:

PULSEWAVE IMAGE (/6)	1 point	0 point
Magnification	Doppler display occupies 50% or more of the image	Doppler display occupies <50% of the image
Angle of insonation	<30 degrees	30 degrees or more
Sweep speed	Doppler spectrum has 4-10 waveforms	Doppler spectrum has < 4 (3 or less) or > 10 waveforms (11 or more)
Sample gate	Large enough to include 3/4th of the vessel diameter	Smaller than 3/4th of the vessel diameter
Appropriate PRF	The waveform fits at least 75% of the pulse wave Doppler scale or PRF (pulse repetition frequency)	The waveform fits < 75% of the pulse wave Doppler scale or PRF
Image quality	Uniform arterial waveforms, no aliasing or background artefacts or fetal breathing movements	Variable arterial waveforms/ aliasing/background artefacts/fetal breathing movements

For MCA: (as above)

PULSEWAVE IMAGE (/6)	1 point	0 point
Magnification	Doppler display occupies 50% or more of the image	Doppler display occupies <50% of the image
Angle of insonation	<30 degrees	30 degrees or more
Sweep speed	Doppler spectrum has 4-10 waveforms	Doppler spectrum has < 4 (3 or less) or > 10 waveforms (11 or more)
Sample gate	Large enough to include 3/4th of the vessel diameter	Smaller than 3/4th of the vessel diameter
Appropriate PRF	The waveform fits at least 75% of the pulse wave Doppler scale or PRF	The waveform fits < 75% the pulse wave Doppler scale or PRF
Image quality	Uniform arterial waveforms, no aliasing or background artefacts or fetal breathing movements	Variable arterial waveforms/ aliasing/background artefacts/fetal breathing movements

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APPENDIX 5: TRUFFLE 2 BIOMARKERS SUB-STUDY: sFlt-1/PlGF RATIO IN SGA AND FGR

SUB-STUDY SUMMARY

TITLE	TRUFFLE 2 biomarkers - sFlt-1/PlGF ratio in SGA and FGR
DESIGN	Nested cohort study
AIMS	To investigate whether the diagnosis and management of LPFGR can be improved with the use of biomarkers
OUTCOME MEASURES	Measuring the sFlt-1/PlGF ratio to: 1. diagnose FGR as an entity distinct from SGA 2. predict which SGA babies will develop FGR 3. inform interval to delivery in SGA and FGR 4. predict perinatal outcomes in SGA and FGR (as per TRUFFLE 2 outcomes) 5. assess the interaction between gestational hypertension and sFlt-1/PlGF in the context of SGA and FGR
POPULATION	As per TRUFFLE 2
ELIGIBILITY	As per TRUFFLE 2
DURATION	As per TRUFFLE 2

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1. INTRODUCTION

1.1 RATIONALE FOR SUB-STUDY

Although there is evidence to suggest that biomarkers such as soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) can predict pregnancy complications in SGA babies close to term (37 weeks gestation) (Sovio *et al.*, 2021) there is currently no published data on the use of these biomarkers in the diagnosis and management of SGA and FGR in late preterm gestations. The consequences of unnecessary late preterm delivery of SGA babies are now more clearly understood (NHS England, 2019) however the ability to identify which SGA babies have FGR and are at risk of adverse perinatal outcomes, and which are constitutionally small and could safely deliver at term remains limited.

We hypothesise that a high sFlt-1/PlGF ratio identifies pregnancies with late-preterm FGR (LPFGR) as opposed to SGA and identifies SGA pregnancies likely to progress to LPFGR. We also hypothesise that high sFlt-1/PlGF ratio can identify pregnancies with SGA and LPFGR at risk of imminent delivery and poor perinatal outcomes. We will collect and store blood for novel biomarker analyses at a later point, with consent, subject to further ethical approvals.

2. SUB-STUDY OBJECTIVES

The aim of this project is to investigate whether the diagnosis and management of LPFGR can be improved with the use of biomarkers. Depending on the outcome of this study the data could support the development of a further study into the diagnosis and management of LPFGR. We aim to determine if maternal serum biomarkers in pregnancy can diagnose FGR in a cohort of SGA pregnancies, predict progression of SGA to FGR, and predict time to delivery and adverse pregnancy outcomes.

3. SUB-STUDY DESIGN

The study will be conducted in London and Milan, the centres of the CI and PI for this study: C Lees (Imperial) and E Ferrazzi (Milan). Data on sFlt-1/PlGF will be collected either by upload of clinical test results (in centres where these tests are normally conducted) or by venous blood draw and analysis. The centres will use techniques for collection, transport and processing of maternal serum biomarkers as recommended by the manufacturer, Roche, which will provide all assays.

Potential participants are women with singleton non-anomalous ongoing pregnancies 32+0 to 36+6 weeks in whom a small for gestational age (SGA) baby is identified or one whose growth has slowed. This is defined as estimated fetal weight or abdominal circumference <10th percentile or decreased by 50 percentiles since an ultrasound scan at 18-32 weeks. Each centre will utilise their local growth charts. Data will be collected on absolute measurements as well as the growth chart used to calculate centiles. Once identified as potential participants they will receive regular monitoring as per the usual standard of care of fetal condition using ultrasound of biometry, cerebral Doppler assessments and cCTG (using Dawes-Redman criteria). This is recommended to be every 14 days. This observational data will be recorded. These participants have SGA babies and are classified as “pre-eligible” in the main RCT.

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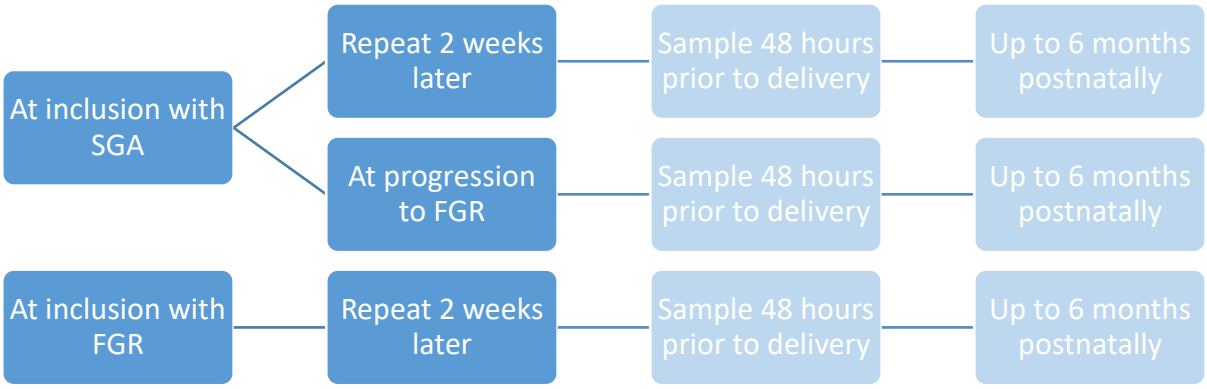
Women will be offered a blood test at the time they are identified as pre-eligible for the TRUFFLE 2 Study (SGA) or eligible for randomisation (FGR). The results of these biomarkers will not be used for clinical decision making for SGA/FGR patients. These tests will be repeated after 2 weeks, or at the time of diagnosis of LPFGR (irrespective of whether the patient wishes to be randomised). They may be offered further tests within the SGA or FGR category.

TIMINGS OF BIOMARKERS
1. Biomarkers at enrolment: Women with a diagnosis of SGA will have blood taken for sFlt-1:PlGF at the time of recruitment into data collection for the TRUFFLE 2 study.
2. Serial biomarkers; Women will be offered at least one further sample 2 weeks after inclusion or at progression to FGR (whichever is sooner).
3. Women may also be offered repeat samples within 48 hours prior to delivery and up to 6 months postnatally.
4. Follow-up: Their pregnancy and perinatal outcomes will be recorded as part of the TRUFFLE 2 Study.



Flowchart showing timing of biomarker blood samples

Serum samples will be collected in a serum collection tube with separator gel by routine venepuncture (5 millilitres). They must be stored upright for at least 30 minutes to allow blood to coagulate, and then centrifuged within 60 min of venepuncture. Serum should be clear and separated from blood clot by the gel layer. Serum should be pipetted into multiple aliquots and stored below -20°C. Frozen samples will be shipped to and measured (blinded for the clinician) by two laboratories (Milan for Italy, Southern, Central and Northern Europe and London for UK centres). Maternal serum levels of sFlt-1 and PlGF (with both levels measured in picograms per milliliter) will be determined by means of the fully automated Elecsys assays for sFlt-1 and PlGF on an electrochemiluminescence immunoassay platform (Cobas e analysers, Roche Diagnostics) and will be used to calculate the sFlt-1/PlGF ratio. These will be processed in batches at least every 6 months.

There will also be an option for sites to participate by submitting lab results for sFlt-1/PlGF measurements if these can be processed within their own laboratory using the Roche assay. For this option participating sites must confirm that the result of the sFlt-1/PlGF ratio is not being used to guide clinical management of LPFGR. These results will be entered into the Castor database, along with other clinical data for the main TRUFFLE 2 Study. Additional consent is not required for these participants.

3.1 SUB-STUDY OUTCOME MEASURES

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Objectives are measuring the sFlt-1/PlGF ratio to:

1. diagnose FGR as an entity distinct from SGA
2. predict which SGA babies will develop FGR
3. inform interval to delivery in SGA and FGR
4. predict perinatal outcomes in SGA and FGR (as per TRUFFLE 2 outcomes)
5. assess the interaction between gestational hypertension and sFlt-1/PlGF in the context of SGA and FGR

Sub-study endpoint as per TRUFFLE 2.

4. PARTICIPANT ENTRY

4.1 PRE-REGISTRATION EVALUATIONS

- Ultrasound scan of fetal growth between 32+0 and 36+6 weeks

4.2 INCLUSION CRITERIA

(All criteria should be fulfilled to be eligible for randomisation)

- Women ≥ 18 years old
- Pregnant with singleton non-anomalous fetuses
- Between 32+0 and 36+6 weeks of gestation
- Estimated fetal weight or abdominal circumference <10th percentile OR decreased by 50 percentiles since an ultrasound scan at 18+0-32+0 weeks

4.3 EXCLUSION CRITERIA

- Indication for immediate delivery required within 48 hours
- Unable to give informed consent
- Preterm prelabour rupture of the membranes (PPROM)
- Suspected placental abruption or antepartum haemorrhage
- Presence of reversed end diastolic flow in the Umbilical Artery

4.4 WITHDRAWAL CRITERIA

Clear contact details for investigators at each unit are clearly presented on the Patient Information Sheet (PIS), and communication between participants and investigators is encouraged throughout the study. Participants should inform any member of the study team of their wish to withdraw consent. All data collected up to the point of withdrawal will be included in the analysis.

5. ADVERSE EVENTS

Adverse events will be reported through the TRUFFLE 2 Study, please see the main protocol for details of this.

6. ASSESSMENT AND FOLLOW-UP

As per the TRUFFLE 2 Study.

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7. STATISTICS AND DATA ANALYSIS

SAMPLE SIZE CALCULATIONS
There are no good data to inform the sample size for this sub-study, therefore the sample size will be formed on the basis of the cardiovascular study and both will run in parallel. Accounting for a 20% drop out rate at 6 months, group size will be 73. We propose to recruit 244 women with SGA of whom (see data from feasibility study, 30% of SGA babies at 32-36+6 weeks gestation will have/develop LPFGR) 73 women will have/develop FGR. We will recruit a cohort of women with healthy pregnancies in the 32+0 – 36+6 week gestation range within a separate ethics application.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

8. REGULATORY ISSUES

8.1 ETHICS APPROVAL
The Study Coordination Centre has obtained approval from the London Riverside Research Ethics Committee (REC) and Health Regulator Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2 CONSENT
Consent to enter the biomarkers study will be sought from each participant at the time of consent to enter the pre-eligible observational data collection part of the TRUFFLE 2 Study. Consent will be obtained with a separate consent form, so as not to interfere with the core TRUFFLE 2 Study, confirming that the patient is also willing to provide a blood sample and whether they consent for serum to be stored for future ethically approved research.

If a site is participating by the option of submitting the results of biomarkers already measured then additional consent is not required. This is because no additional phlebotomy is taking place, and patients have already consented to clinical information being collected for the TRUFFLE 2 Study.

8.3 CONFIDENTIALITY
The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

8.4 INDEMNITY
Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

8.5 SPONSOR
Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

8.6 FUNDING
This study is undertaken by those centres that are resourced to take blood samples, and can spin, store and transport them to the processing centres. Roche will provide assays and

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sufficient funding for the sample analysis, but do not have rights over the study findings and data. This study will not be funded by the NIHR HTA TRUFFLE 2 Grant.

8.7 AUDITS

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Frame Work for Health and Social Care Research.

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Dr Bronacha Mylrea-Foley at Imperial College London.

10. PUBLICATION POLICY

As for the TRUFFLE-2 study, in line with NIHR's Open Access Policy.

11. REFERENCES

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3. NHS England (2019). Saving Babies' Lives Version Two: A care bundle for reducing perinatal mortality. https://www.england.nhs.uk/publication/saving-babies-lives-version-two-a-care-bundle-for-reducing-perinatal-mortality/

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APPENDIX 6: TRUFFLE 2: HEALTH ECONOMIC EVALUATION

A prospective health economic evaluation, conducted from an NHS perspective, will be integrated into the trial design. The economic evaluation will estimate the difference in the cost of resource inputs used by participants in the two arms of the TRUFFLE2 trial. It will thereby allow comparisons of economic costs and health consequences to be made between the alternative methods of timing delivery in women with singleton ongoing pregnancies at 32-36 weeks in whom a small for gestational age baby is identified or one whose growth has slowed. The economic assessment method will, as far as possible, adhere to the recommendations of the NICE Reference Case (NICE, 2013). Primary research methods will be followed to estimate the costs of the alternative options for timing delivery, framed by method and characteristics of induction of labour, clinical monitoring, the mode of delivery and supplementary interventions. Broader health service resource utilisation will be captured through two principal sources: (1) resource inputs associated with the duration and intensity of antenatal, intrapartum, postnatal and neonatal care, based on standard criteria for level of care, as well as maternal and neonatal complications, will be extracted from the CASTOR data collection system; and (2) patient questionnaires administered at 6, 12, 18 and 24 months post-randomisation. Unit costs for health resources will largely be derived from local and national sources and estimated in line with best practice. Primary research using established accounting methods may also be required to estimate unit costs. Costs will be standardised to current prices where possible. Multiple imputation methods will be used to impute missing data and avoid biases associated with complete case analysis (Rubin, 2004). The economic evaluation will be framed as a cost-effectiveness analysis with results expressed in terms of incremental cost per additional case of poor condition at birth and/or fetal or neonatal death or major morbidity avoided (short term outcome) and incremental cost per additional case of adequate neurodevelopmental outcome (long term outcome). Bivariate regression of costs and health outcomes will be conducted to generate within-trial estimates of incremental cost-effectiveness (Glick et al., 2014). We shall use non-parametric bootstrap estimation to derive 95% confidence intervals (CIs) for mean cost differences between the trial groups and to calculate 95% CIs for incremental cost- effectiveness ratios (Efron and Tibshirani, 1993; Glick et al., 2014). A series of sensitivity analyses will be undertaken to explore the implications of uncertainty on the incremental cost-effectiveness ratios and to consider the broader issue of the generalisability of the study results. One such sensitivity analysis will involve restricting the analyses to complete cases, i.e. those with complete cost and outcome data for the two alternative time horizons. In the baseline analysis, and for each sensitivity analysis, cost-effectiveness acceptability curves will be constructed using the net-benefits approach (Fenwick et al., 2004). Heterogeneity in the trial population will be explored by formulating net-benefit values for trial participants from the observed costs and effects and then constructing a regression model with an intervention variable and pre-specified covariates such as sex. The magnitude and significance of the coefficients on the interactions between the covariates and the intervention variable will provide estimates of the cost-effectiveness of the alternative methods of timing delivery options by participant subgroup.

References

1. Efron B, Tibshirani R. *An introduction to the bootstrap*. New York: Chapman & Hall; 1993.
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3. Glick H, Doshi JA, Sonnad SS, Polsky D. *Economic evaluation in clinical trials*. Second edition. Oxford: Oxford University Press, 2014.
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APPENDIX 7: TRUFFLE 2 CARDIOVASCULAR SUB-STUDY: MATERNAL HAEMODYNAMICS IN SGA AND FGR

SUB-STUDY SUMMARY

TITLE	TRUFFLE 2 cardiovascular sub-study: maternal haemodynamics in SGA and FGR
DESIGN	Nested cohort study
AIMS	To investigate the association between maternal haemodynamic parameters, fetal status and neonatal outcome. To evidence a link between maternal haemodynamic status during pregnancy and maternal outcome.
OUTCOME MEASURES	Measuring maternal cardiovascular function to: 1. diagnose FGR as an entity distinct from SGA 2. predict which SGA babies will develop FGR 3. inform interval to delivery in SGA and FGR 4. predict perinatal outcomes in SGA and FGR (as per TRUFFLE 2 outcomes) 5. assess the interaction between haemodynamic markers and sFlt-1/PlGF biomarkers
POPULATION	As per TRUFFLE 2
ELIGIBILITY	As per TRUFFLE 2
DURATION	As per TRUFFLE 2

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1. INTRODUCTION

1.1 RATIONALE FOR SUB-STUDY

Women pregnant with FGR fetuses show high total peripheral resistance (TPR) and suboptimal rise of cardiac output (CO), both at latent and clinical stages of disease (Stott, 2016; Di Martino, 2019). Assessment of maternal haemodynamics is useful to identify pregnancies at risk for FGR already at early gestation (Stott, 2016). Longitudinal studies from preconception show high TPR and low CO in those pregnancies which go on to develop FGR (Foo, 2018).

Published data describes the aetiological role of maternal cardiovascular function in fetal growth and, by extension of this concept, in stillbirth. If maternal cardiovascular function deteriorates in advance of fetal Doppler deterioration in FGR, reflecting worsening fetal condition, this could be an avenue to potential therapy for FGR, currently lacking. Longitudinal maternal cardiovascular assessment in FGR could improve identification of those fetuses who could benefit from early delivery or conservative management.

We have established the differences in cardiovascular function in pre-eclampsia with and without fetal growth restriction (Tay, 2018). Maternal CO and TPR correlate with maternal uterine artery and fetal umbilical artery Doppler parameters in a dose-dependent manner suggesting that changes in maternal cardiovascular function may drive fetal Doppler changes (Tay, 2018).

We will measure maternal cardiac output and TPR at diagnosis of SGA and LPFGR and track changes in the mother's cardiovascular system through pregnancy alongside maternal biomarkers and fetal Doppler to establish the chronology of change in fetal compromise. This will establish the relationship between maternal hemodynamic function and fetal circulation in LPFGR. We will also measure change in maternal cardiovascular function over time, into the postnatal period. Using different techniques for maternal cardiovascular assessment, depending on centre preferences, parameters are then normalised with respect to gestational week and can be compared across centres (Masini, 2018) including inert gas breathing (Tay, 2018), pulse waveform analysis and ultrasonic cardiac output monitoring (Masini, 2018), impedance cardiography (Gyselaers, 2018) and bioreactance cardiac output monitoring (Vinayagam, 2017) in relation to FGR and stillbirth. Cardiovascular measurements will be undertaken at enrolment, at progression to FGR or 2 weeks later (whichever is sooner), at delivery and 6 months postnatal.

The established sub-optimal maternal rise in CO in a pregnancy affected by FGR (Stott, 2016) is hypothesised to contribute to poor fetal growth through inadequate perfusion of the placenta, high TPR being a compensatory response though one whose effects are deleterious for the mother. In pre-eclampsia, cardiac function can be abnormal for at least 4 years after pregnancy (Breetveld, 2017), postnatal changes in cardiac function have not been characterised in LPFGR. These may identify women at risk of later life cardiovascular disease.

2. SUB-STUDY OBJECTIVES

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Objectives are to investigate the correlation between maternal haemodynamic status, fetal status and neonatal outcome, and to evidence a link between maternal haemodynamic status during pregnancy and maternal outcome. In addition we aim to explore the relationship between biomarkers and cardiovascular function within SGA and FGR.

We expect that mothers with worse haemodynamic adaptation are more likely to carry more compromised babies (and therefore progress to FGR), are more likely to have smaller babies and worse neonatal and long-term outcome and are more likely to suffer from hypertension, pre-eclampsia/HELLP syndrome, and have worse maternal outcomes.

3. SUB-STUDY DESIGN

Centres participating in the TRUFFLE 2 Study will be invited to join the cardiovascular sub-study. Sites will need to be able to perform non-invasive haemodynamic assessment. Any commercially available validated method or instrument for non-invasive haemodynamic maternal assessment can be used. Studies show broad equivalence of the different instruments and techniques as long as the results can be converted to MoMs for gestational week (ie normal ranges for the equipment exist).

Potential participants are women with singleton non-anomalous ongoing pregnancies 32+0 to 36+6 weeks in whom a small for gestational age (SGA) baby is identified or one whose growth has slowed. This is defined as estimated fetal weight or abdominal circumference <10th percentile or decreased by 50 percentiles since an ultrasound scan at 18-32 weeks. These participants have SGA babies and are classified as “pre-eligible” in the main RCT.

Non-invasive haemodynamic evaluation of the mother will be offered at time they are identified as pre-eligible for the TRUFFLE 2 Study (SGA) or eligible for randomisation (FGR). The results of these assessments will not be used for clinical decision making for SGA/FGR patients. These assessments will be repeated after 2 weeks, or at the time of diagnosis of LPFGR (irrespective of whether the patient wishes to be randomised). They may be offered further tests within the SGA or FGR category.

1. Cardiovascular function at enrolment: Women with a diagnosis of SGA will have a cardiovascular assessment at the time of recruitment into data collection for the TRUFFLE 2 study.
2. Serial cardiovascular function; Women will be offered at least one further assessment 2 weeks after inclusion or at progression to FGR (whichever is sooner).
3. Women may also be offered repeat assessments within 48 hours prior to delivery and up to 6 months postnatally.
4. Follow-up: Their pregnancy and perinatal outcomes will be recorded as part of the TRUFFLE 2 Study.

Please note that the timing of haemodynamic assessment should coincide with the timing of biomarkers if the participant is taking part in both sub-studies. If, at study inclusion, the woman does not wish to have her haemodynamics assessment performed that day and wishes to return at a later date, she may do so as soon as possible after inclusion. If she is also willing to participate in the biomarkers sub-study, we would aim to perform both haemodynamics and biomarkers at the same time.

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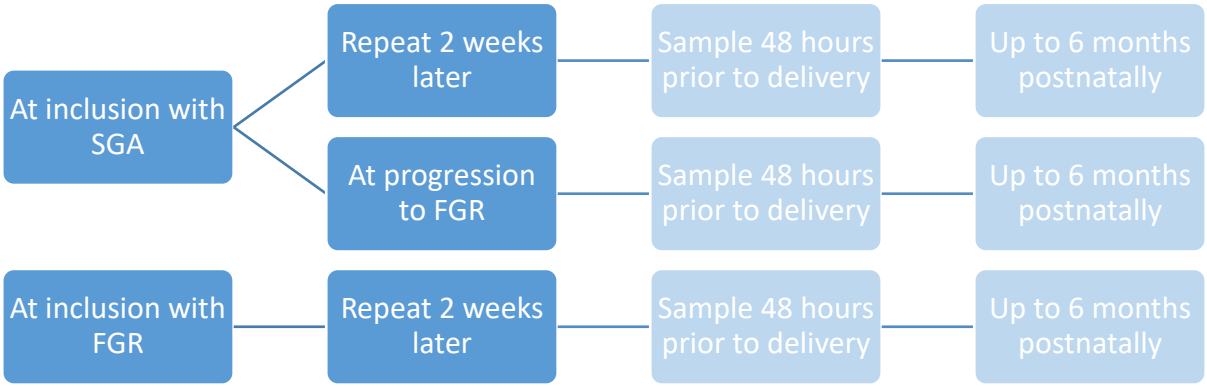

Flowchart showing timing of maternal haemodynamic assessment

Participating sites will enter the results of these measurements into the Castor Electronic Data Collection database, along with other clinical data for the main TRUFFLE 2 Study.

3.1 SUB-STUDY OUTCOME MEASURES

Measuring maternal cardiovascular function to:

1. diagnose FGR as an entity distinct from SGA
2. predict which SGA babies will develop FGR
3. inform interval to delivery in SGA and FGR
4. predict perinatal outcomes in SGA and FGR (as per TRUFFLE 2 outcomes)
5. assess the interaction between haemodynamic markers and biomarkers

Sub-study endpoint as per TRUFFLE 2.

4. PARTICIPANT ENTRY

4.1 PRE-REGISTRATION EVALUATIONS

- Ultrasound scan of fetal growth between 32+0 and 36+6 weeks

4.2 INCLUSION CRITERIA

(All criteria should be fulfilled to be eligible for randomisation)

- Women ≥ 18 years old
- Pregnant with singleton non-anomalous fetuses
- Between 32+0 and 36+6 weeks of gestation
- Estimated fetal weight or abdominal circumference <10th percentile OR decreased by 50 percentiles since an ultrasound scan at 18+0-32+0 weeks

4.3 EXCLUSION CRITERIA

- Indication for immediate delivery required within 48 hours
- Unable to give informed consent
- Preterm prelabour rupture of the membranes (PPROM)
- Suspected placental abruption or antepartum haemorrhage

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- Presence of reversed end diastolic flow in the Umbilical Artery

4.4 WITHDRAWAL CRITERIA

Clear contact details for investigators at each unit are clearly presented on the Patient Information Sheet (PIS), and communication between participants and investigators is encouraged throughout the study. Participants should inform any member of the study team of their wish to withdraw consent. All data collected up to the point of withdrawal will be included in the analysis.

5. ADVERSE EVENTS

Adverse events will be reported through the TRUFFLE 2 Study, please see the main protocol for details of this.

6. ASSESSMENT AND FOLLOW-UP

As per the TRUFFLE 2 Study.

7. STATISTICS AND DATA ANALYSIS

SAMPLE SIZE CALCULATIONS
Sample size calculated from Tay, 2018 using Cardiac Output in women FGR-affected pregnancies, mean and sd 5.56 and 1.06 l/min., with 58 patients per group, 90% power with alpha 0.05 to detect a difference of 0.64l/min. Accounting for a 20% drop out rate at 6 months, group size will be 73. We propose to recruit 244 women with SGA of whom (see data from feasibility study, 30% of SGA babies at 32-36+6 weeks gestation will have/develop LPFGR) 73 women will have/develop FGR. We will recruit a cohort of women with healthy pregnancies in the 32+0 – 36+6 weeks gestation range within a separate ethics application.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

8. REGULATORY ISSUES

8.1 ETHICS APPROVAL

The Study Coordination Centre has obtained approval from the London Riverside Research Ethics Committee (REC) and Health Regulator Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2 CONSENT

Consent to enter the cardiovascular sub-study will be sought from each participant at the time of consent to enter the pre-eligible observational data collection part of the TRUFFLE 2 Study. Consent will be obtained with a separate consent form, so as not to interfere with the core TRUFFLE 2 Study, confirming that the patient is also willing to undergo serial maternal haemodynamics assessment.

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8.3 CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

8.4 INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

8.5 SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

8.6 FUNDING

This study is undertaken by those centres that are resourced to perform maternal haemodynamics assessment. This study will not be funded by the NIHR HTA TRUFFLE 2 grant.

8.7 AUDITS

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Frame Work for Health and Social Care Research.

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Dr Bronacha Mylrea-Foley at Imperial College London.

10. PUBLICATION POLICY

As for the TRUFFLE-2 study, in line with NIHR's Open Access Policy.

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