Non-CTIMP Study Protocol

I-TEST: Novel Biomarkers in Pregnancy for Early Prediction of Stillbirth

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CONTENTS

LIST OF ABBREVIATIONS

ACCORD	Academic and Clinical Central Office for Research & Development - Joint office for The University of Edinburgh and Lothian Health Board
AWI	Adults With Incapacity
BP	Blood Pressure
CI	Chief Investigator
CRF	Case Report Form
eCRF	Electronic Case Report Form
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
ICH GCP	International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice
ISF	Investigator Site File
PI	Pulsatility Index
PIS	Participant Information Sheet
PSV	Peak systolic velocity
REC	Research Ethics Committee
SOP	Standard Operating Procedure

1 INTRODUCTION

1.1 BACKGROUND

Every 16 seconds one baby is stillborn. That amounts to more than two million stillborn babies globally every year (1). Stillbirths have long-lasting personal and psychological consequences for parents and families, as well as substantial costs for wider society (2). Stillbirth is the endpoint of a number of different processes that involve the mother, baby, or the placenta – or a combination of the three (3). The lack of methods to assess gestational development in utero limits our ability to predict the risk of stillbirth. Today 25-50% of stillbirths are unexplained - meaning that no conditions that affect the mother, baby, or placenta that could contribute to the baby's death are identified.

The eye provides a unique "window" to detect microvascular changes through analysis of images showing the retinal and choroidal microvascular networks. Increasing evidence supports structural and functional changes in retinal blood vessels as a biomarker for early identification of pathological conditions including neurodegenerative diseases, diabetes, renal disease and stroke (4-7). A handful of studies have reported changes in the retinal vasculature in association with pregnancy complications including pre-eclampsia (8) and reduced fetal growth (9). This raises the exciting potential for retinal imaging in pregnancy to be used for early detection of pregnancy pathologies, allowing early preventative measures. Use of retinal imaging is attractive as it is non-invasive, quick to perform (<15 minutes) and can be done at scale. However, during pregnancy there are dramatic changes to the circulation and vasculature. Little is known about the natural variation of the retinal vasculature across this short dynamic timeframe.

Moreover, pregnancy pathologies can be associated with significant long-term maternal health consequences: women who suffer from pre-eclampsia have an over 2-fold increased lifelong risk of stroke, acute coronary syndrome, and young-onset dementia, an over 7-fold increase in chronic kidney disease risk, and an over 4-fold increased risk of cardiovascular death (10,11). There is increasing awareness that cardiovascular risk stratification should include information about adverse pregnancy outcomes (12). However, there is a knowledge gap with regards to the pathological link between vascular dysfunction in pregnancy and later cardiovascular health, and how to identify and monitor those most at risk (in order to effectively target preventative intervention).

We will develop and test the use of novel biomarkers derived from retinal imaging as an innovative, non-invasive measure of maternal vascular responses to pregnancy, fetal health, placental insufficiency and long-term maternal cardiovascular health. We will identify new **retinal biomarkers** for integration into models that are predictive of the risk of stillbirth and future maternal cardiovascular disease.

1.2 RATIONALE FOR STUDY

Our main goal is to test the use of novel biomarkers derived from retinal imaging for early detection of pregnancy complications and integration into models that are predictive of the risk of stillbirth and future maternal cardiovascular disease. We aim to achieve this by establishing two pregnancy cohorts – cohort 1 with longitudinal multimodal retinal imaging in early and late pregnancy, and cohort 2 with cross-sectional multimodal imaging in late pregnancy. Our proposal will fill the knowledge gap about the natural variation of the retinal vasculature across pregnancy. We will develop a suite of novel retinal biomarkers suitable for inclusion in prediction models of stillbirth and future maternal cardiovascular disease, which will be developed and validated in our cohort. We will assess the viability of these retinal biomarkers at critical time points in pregnancy when interventions to prevent or reduce the risk of stillbirth are available.

In high income countries, aspirin commenced prior to 16 weeks of gestation reduces the risk of placental dysfunction including early onset pre-eclampsia, preterm birth and stillbirth. We currently only recommend aspirin prophylaxis for women with higher risk pregnancies because there are also risks associated with aspirin treatment. The most effective first trimester risk prediction algorithms incorporate uterine artery Doppler and biomarkers like PLGF or PAPP-A assessed at 12 weeks in alignment with

the aneuploidy screening window. The associated costs and resources of additional sonographer time and sample processing have limited implementation of these algorithms. Retinal imaging derived markers may be complementary or superior to existing variables used for pregnancy risk assessment and retinal biomarkers are an attractive alternative for scale up and implementation of streamlined first trimester risk assessment.

In low income countries, pregnant women often do not present for their first antenatal visit until around 20 weeks of pregnancy. In this setting, where resources are limited meaning ultrasound and expensive blood tests are not available, retinal imaging may be a useful option.

Similarly, a risk assessment at around 36 weeks would facilitate personalised recommendations for mode and timing of birth to optimally reduce stillbirth risk. No clinically applicable algorithms yet exist for this time point. In our two cohorts, we will be able to assess both the clinical utility of longitudinal change in retinal markers in pregnancy and cross-sectional variation at 36 weeks gestation in order to determine the most informative measure for use in later pregnancy risk assessment.

We have therefore selected 12, 20 and 36 weeks gestation as our timepoints for antenatal retinal imaging.

Persistent abnormal patterns of maternal cardiac remodelling and elevated risks of essential hypertension have been identified one and two years, respectively, following pre-eclamptic pregnancies (13). This subclinical cardiovascular phenotype may represent the link between the disordered maternal vascular responses seen in pregnancy complications and increased long-term cardiovascular disease risk. If retinal changes associated with disordered maternal vascular responses to pregnancy persist into the postpartum period, these may act as an original biomarker of elevated cardiovascular vulnerability which could guide risk factor modification to optimise long-term health. This would be particularly timely, as interest is growing in directed postnatal therapeutic approaches to modify long-term cardiovascular risk (14-15).

We have therefore selected 12 (+ / - 6) months postnatal as our timepoint for follow-up retinal imaging.

2 STUDY OBJECTIVES

2.1 OBJECTIVES

2.1.1 Primary Objective

To assess variability in the key retinal biomarkers at 36 weeks gestation and their longitudinal trajectories over time (between 12 and 36 weeks' gestation, between 20 and 36 weeks' gestation, and between 36 weeks' gestation and 12 months postpartum).

2.1.2 Secondary Objectives

To collect:

Blood samples at 36 weeks' gestation and 12 (+ / - 6) months postpartum for storage and measurement of biomarkers of placental dysfunction or stillbirth (for antenatal samples), and maternal vascular dysfunction

Ultrasound image data of fetal development at 36 weeks.

Data on key maternal and neonatal outcomes.

Physiological data on maternal cardiovascular function at 12 (+ / - 6) months postpartum, from non-invasive tests.

Urine samples at 12 (+ / - 6) months postpartum for storage and measurement of proteinuria and other potential biomarkers of maternal cardiovascular risk.

2.2 ENDPOINTS

2.2.1 Primary Endpoint

Collection of a variety of maternal retinal images and fetal biomarker measurements during pregnancy for integrating into a mathematical model predicting stillbirth.

2.2.2 Secondary Endpoints

Collection of a variety of maternal retinal and cardiovascular metrics during the postpartum period for integrating into a mathematical model predicting adverse long-term cardiovascular health outcomes.

Bioresource suitable for measurement of biomarkers of placental dysfunction, stillbirth and postnatal maternal cardiovascular dysfunction.

3 STUDY DESIGN

We propose 2 parallel cohorts:

a) Longitudinal cohort with serial multimodal retinal imaging at 12 and 36 weeks or at 20 and 36 weeks in unselected pregnancies (cohort 1).

b) Cross-sectional cohort with multimodal retinal imaging in late pregnancy obtained at 36 weeks, in a cohort enriched with higher risk pregnancies recruited via the fetal growth and hypertension monitoring services (cohort 2).

All participants will be invited to a single follow-up visit at 12 (+ / - 6) months postpartum, for repeat retinal imaging and cardiovascular assessment.

4 STUDY POPULATION

4.1 NUMBER OF PARTICIPANTS

Cohort 1: 330 pregnant women with singleton pregnancy recruited at 12 weeks' gestation (+ / - 3 weeks) or 20 weeks' gestation (+ / - 3 weeks).

Cohort 2: 225 pregnant women with singleton pregnancy recruited after 23 weeks' gestation, with retinal imaging at 36 (+ / - 3) weeks gestation.

4.2 INCLUSION CRITERIA

Age 16-50 years.

Able to give informed consent.

Singleton pregnancy.

12 (+ / - 3) weeks gestation or 20 (+ / - 3) weeks gestation – cohort 1

>23 weeks gestation – cohort 2

Living in Lothian area

4.3 EXCLUSION CRITERIA

Women who are not pregnant.

Women aged under 16 years or over 50 years.

Women who are classified as Adults with Incapacity (AWI) as determined by midwife, GP or research team.

Multiple pregnancy.

4.4 CO-ENROLMENT

Women will be able to enrol into other studies if they choose to do so.

5 PARTICIPANT SELECTION AND ENROLMENT

5.1 IDENTIFYING PARTICIPANTS

Women may be identified in a number of ways:

- A study leaflet included in the antenatal booking packs, which are sent out centrally once a pregnancy has been confirmed. This will include a QR code which links to the study website where the participant can read the PIS.
- At their first antenatal booking appointment, which is typically between 6-12 weeks' gestation, in a community midwife setting, midwives can provide a patient information sheet and sticker containing a QR code which links to the study website where the pregnant women can read the PIS.
- At the 12-week or 20 week antenatal booking scan.
- Advertising the study on the Edinburgh Pregnancy Research Team social media (X, Facebook, Instagram), in posters and leaflets all of which will contain QR codes and study weblinks. Women who have had a pregnancy confirmed will be able to self-identify and visit the study website and contact the team to express interest.
- Targeted Advertisements on Social Media and other Social Media content will be created in conjunction with and administered by an external digital marketing company called Nativve https://www.nativve.com/. Nativve have been added as an approved supplier to the University and we have experience working with them for another pregnancy study.
- By sending information about the study to women who are participating in another pregnancy study 'Born in Scotland' who have given specific consent to be approached to participate in other studies.
- A member of the clinical research team will also identify potential participants attending antenatal services including routine third trimester growth scans, antenatal clinics, antenatal wards, the maternity day assessment unit, and the small baby clinic. Any woman who is interested in taking part will be invited to meet a member of the research team during their routine clinical visit.
- Posters advertising the study will be displayed in staff areas at the Royal Infirmary of Edinburgh, and within the University of Edinburgh in areas frequented by staff and students.
- We will also disseminate information about the study through email to NHS Lothian and University of Edinburgh staff mailing lists.

Participants who have taken part in I-Test under study protocol version 3.0 or earlier, and who have completed their pregnancy in the last 12 (+/- 6) months, may be re-contacted by email or telephone to invite them to a follow-up assessment (as detailed in section 6.2). It will be made clear to participants that returning for follow-up is optional and that they have the right to decline without giving any reason. Information about this follow-up visit will also be included on the study website.

5.2 CONSENTING PARTICIPANTS

All procedures will be explained to each participant either by a member of the research team, or by a video on the website and written information will be provided on a detailed participant information sheet (PIS). Prior to providing written consent, all participants will be given ample time to consider whether or not they would like to take part and to ask questions relating to the research. Additionally, it will be made clear both verbally and in writing that all volunteers have the right to withdraw at any time, without giving any reason, and this will not affect the care they receive. Informed consent will be sought from the

participant themselves as only healthy adults will be recruited. Consent may be given online – via a uniquely generated url which links to the consent form on the REDCap database. Consent may also be obtained via paper on the day of the study visit.

For participants who have given consent to I-Test under study protocol version 3.0 or earlier version and are still participating in the study, re-consent may be sought under the up-to-date protocol (to cover long-tern follow-up assessments as detailed in section 6.2). For participants who have completed their pregnancy within the last 12 (+/-6) months, and who were consented under protocol version 3.0 or earlier, re-consent will be sought under the up-to-date protocol if they take up an invitation to a follow-up visit.

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 or research midwives. If withdrawal occurs, the primary reason for withdrawal will be documented in the participant's case report form, if possible. The participant will have the option of withdrawal from:

(i) all aspects of the study but continued use of data and samples collected up to that point. To safeguard rights, the minimum personally-identifiable information possible will be collected.
(ii) all aspects of the study but continued use of data and samples collected up to that point and also routine data collection at birth from the electronic health care record. To safeguard rights, the minimum personally-identifiable information possible will be collected.
(iii) all aspects of the study and all data and samples collected up to the point will be withdrawn and we will not collect any further data or samples.

Women will be able to withdraw from the study at any time by emailing or telephoning a member of the research team, whose contact details will be provided on patient facing materials, or via the website contact us form.

6 STUDY ASSESSMENTS

6.1 STUDY ASSESSMENTS

Retinal imaging

This will involve comfortable and convenient examination for the participants, capturing crucial types of eye measurements and retinal imaging. Conducted at baseline in cohort 1 (12 (+ / - 3) weeks or 20 (+ / - 3) weeks) and at 36 (+ / - 3) weeks in all participants, women will be invited to have retinal imaging and measures of the eye using the following devices.

The iCare tonometer:

- A handheld device which uses an induction based rebound method (assessing the deceleration and rebound time of a small, lightweight probe, which makes brief contact with the cornea) which allows intraocular pressure (IOP) to be measured accurately.

The Oculus Myopia Master which produces 3 metrics of the eye:

- Auto-Refractometer that that calculates refractive error.
- Keratometer to determine the curvature of the cornea.
- Axial Length which is the distance between the anterior surface of the cornea and the fovea.

Heidelberg Engineering SPECTRALIS platform capturing 3 types of retinal imaging:

- a 2D infra-red image of the fundus (surface of the retina showing blood vessels, optic nerve head and macula) (16).

- Optical coherence tomography (OCT) (3D data capturing the retinal layers and deeper lying choroidal vasculature) (17).
- Optical coherence tomography angiography (OCTA) (smaller capillary level vessels closer to inner retinal surface) (18).

The Canon CR-DGi non-mydriatic fundus camera:

- a 2D colour photograph of the fundus (surface of the retina showing blood vessels, optic nerve head and macula).

Members of our team in Edinburgh Imaging work to standard operating procedures (SOPs) for capture across our existing portfolio of retinal research, thus helping to ensure eye data is fit for analysis.

Ultrasound measurements

At 36 (+ / - 3) weeks we may conduct as supplementary assessments:

- Maternal ultrasound assessment: Uterine artery Doppler measurements may be made and the pulsatility index (PI) from both uterine arteries recorded. Maternal ophthalmic artery Doppler measurements may be made, with first peak systolic velocity (PSV), second PSV, pulsatility index (PI), and ratio of second to first PSV being recorded for the right eye, left eye, and again right and then left eye.
- Fetal Doppler waveforms may be collected on the middle cerebral artery (PI and PSV), Ductus Venosus ('a' wave presence or absence, peak velocity index for veins, pulsatility index for veins) and Umbilical Artery PI.
- In addition to the standard clinical measures the following research images may be captured using a handheld Clarius ultrasound system: the longest axis of the placenta from the closest maternal window (left, right or anterior), a long axis slice of fetal liver at maximum diameter and length and an axial slice of the thorax with the four chamber view and bilateral lung fields at the level of the AV valves.

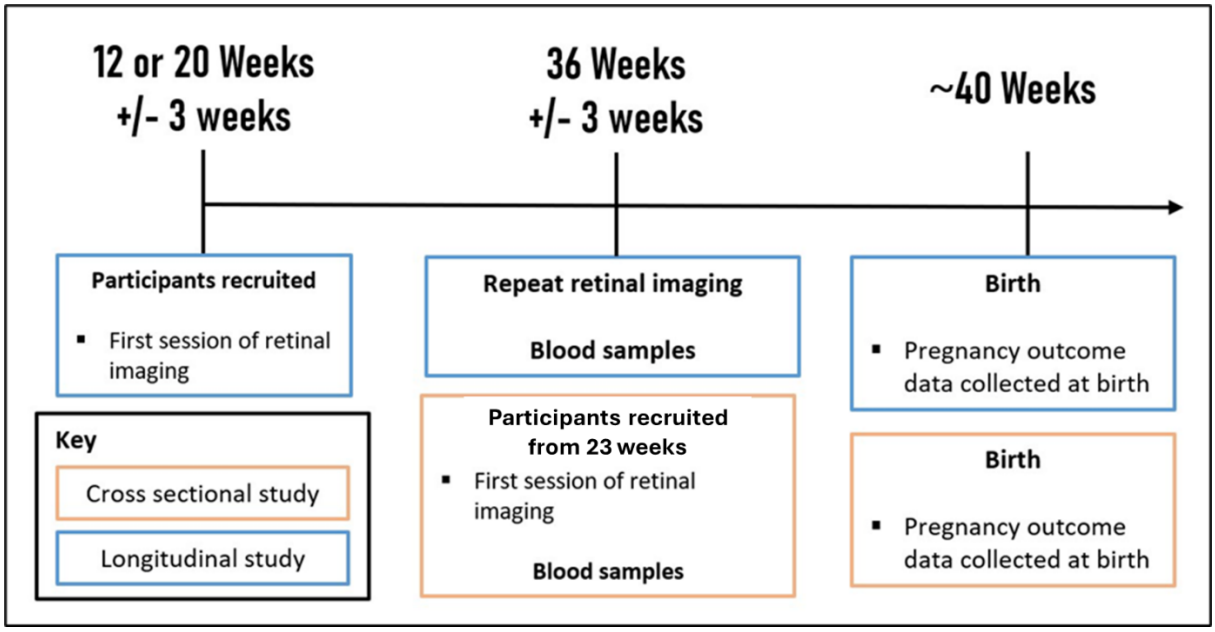
If a 36-week growth ultrasound scan is scheduled as part of clinical care, relevant data may be taken from the clinical scan.

At 36 (+ / - 3) weeks we will collect and store blood samples for measurement of biomarkers of placental dysfunction, maternal vascular dysfunction, and stillbirth. Participants may decline bloods and still take part in the study.

Key maternal demographics and maternal and neonatal outcomes will be collected at birth from the electronic health record.

Figure 1 below demonstrates both streams in chronological form.

Figure 1 Antenatal study visits overview



6.2 LONG TERM FOLLOW UP ASSESSMENTS

Participants from both cohorts may be invited to a follow-up study visit at 12 (+ / - 6) months postpartum. Contact to invite participants to follow-up will be by telephone or email, and it will be made clear to participants that returning for follow-up is optional and that they have the right to decline without giving any reason. Information about this follow-up visit will also be included on the study website.

The follow-up assessment will comprise retinal imaging of both eyes, as detailed in Section 6.1. We may also carry out the following maternal cardiovascular assessments:

- Measurement of height and weight
- Heart rate and systolic and diastolic blood pressure
- Echocardiography, including measurement of cardiac output, left ventricular geometry, and left ventricular systolic and diastolic function
- Pulse wave velocity: aortic central pulse wave velocity assessed with a tonometry-based device placed sequentially over the carotid and femoral artery in conjunction with simultaneous ECG recording. The distance between points of pulse wave detection will be measured manually to allow calculation of central pulse wave velocity. The measurement will be repeated three times, or until at least two measures are within 0.5m/s.
- Verbal self-report of smoking status and family history of cardiovascular disease (angina or myocardial infarction in a first degree relative aged <60 years)
- 24-hour ambulatory blood pressure and arterial stiffness measurement, using a validated wearable ambulatory blood pressure and arterial stiffness monitor which takes measurements every 30 minutes for 24 hours, from which systolic, diastolic and mean arterial pressure, pulse wave velocity, and augmentation index will be calculated. This will allow us to identify loss of nocturnal blood pressure “dipping” (a pattern associated with increased cardiovascular disease risk). The monitor will be given to participants to take home and return of equipment arranged by post.

We will collect and store blood samples for measurement of biomarkers of cardiovascular dysfunction. We will also collect a urine sample for analysis of proteinuria and for storage and future measurement of other potential biomarkers of cardiovascular dysfunction. Participants may decline collection of biosamples and still take part in study follow-up.

In addition, we will seek consent from participants to collect outcome data on cardiovascular risk factors (including hypertension, diabetes, dyslipidaemia) and future cardiovascular events through lifelong data linkage to GP and hospital records.

6.3 STORAGE AND ANALYSIS OF SAMPLES

We will collect venous blood samples (up to 30 mls) at the 36-week antenatal study visit and at the 12 month postnatal follow-up visit. Samples will be processed by a member of the study team and stored in the Edinburgh Reproductive Tissue Biobank within the Queen's Medical Research Institute (QMRI).

Analysis of blood samples retained in the Edinburgh Reproductive Tissue Biobank will include measurement of placental growth factor (PlGF) in antenatal samples, and measurement of glucose, lipid profile, high sensitivity cardiac troponin, brain natriuretic peptide (BNP) and renal function in third trimester and postnatal samples.

The clinical significance of an isolated raised troponin, BNP or glucose in pregnancy and/or in the absence of clinical symptoms is uncertain, therefore abnormal results will not be reported to participants in the case of these measures. In contrast, if significant abnormalities in renal function or lipid profile (which would merit follow-up or consideration of further investigation) is identified, these will be communicated both to participants and their GP.

Urine samples (up to 50mls) will be collected at the 12-month postnatal follow-up visit. An aliquot will be taken to be processed and stored in the Edinburgh Reproductive Tissue Biobank. The remaining sample will be analysed for proteinuria (albumin-creatinine ratio) by NHS Lothian laboratories.

The Edinburgh Reproductive Tissue Biobank will process and store the samples according to their standard operating procedures (SOPs). Biological samples will be anonymised using the unique study identifier used for participant data (allowing samples to be matched with the data), stored in the Edinburgh Reproductive Tissue Biobank ultra-low temperature freezers within the QMRI, and destroyed after ten years.

7 DATA COLLECTION

A unique study identifier will be allocated to each participating woman at recruitment and this unique number will be used for data collection within the study. Identifiers are stored separately from the main data tables and only delegated members of the team will be granted access to these identifiers.

Data will be collected by a delegated member of the research team using an electronic case report form (eCRF). Data collected includes: a) socio-demographics, medical and obstetric history b) details of the current pregnancy including pregnancy complications. c) birth outcomes including birthweight, gestation at birth, baby sex, mode of birth, indication for delivery, admission to the neonatal unit, respiratory distress, Apgar scores and umbilical cord pH.

Ongoing collection of long-term follow-up data will be through linkage to primary and secondary care healthcare records, and will include diagnostic, laboratory, and prescribing data relating to cardiovascular risk factors (including hypertension, diabetes, and dyslipidaemia). We will also collect outcome data relating to cardiovascular events and diagnoses including stroke, acute coronary syndrome, heart failure, renal disease, dementia, and cardiovascular death.

7.1 Source Data Documentation

Source data is defined as all information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. Source data are contained in source documents.

Source documents are original documents, data and records where source data are recorded for the first time.

The Investigator will maintain source documents for each patient in the study, consisting of mother and baby case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, and the results of any other tests or assessments. All information relevant

to a participant's general medical history on eCRFs must be traceable to these source documents in the patient's case notes.

7.2 Case Report Forms

Electronic case report forms will be utilised.

8 DATA MANAGEMENT

8.1 Personal Data

The following personal data will be collected as part of the research: demographic, clinical and personal data including CHI-number; postcode; ethnicity; contact telephone number; email address; date of birth; estimated delivery date.

The CI is the custodian for the study data.

8.2 Data Information Flow

Data will be extracted from the electronic health record of mother and baby and after anonymisation, clinical details entered into the study database. Each patient will be allocated a study number that will be used to identify the patient; no personal identifiable information will be made accessible to anyone outside delegated members of the clinical research team. Data will be filled in at each study visit.

8.3 Data Storage

Personal data will be stored by the research team on a password protected secure NHS computer in a locked office at Royal Infirmary of Edinburgh.

All local paper files containing personal data will be held in filing cabinets in NHS offices that will be locked when unattended. Access to the study documents will be by the study team only.

Research data collected will be stored on secure servers hosted by the University of Edinburgh that require user authentication.

8.4 Data Retention

Participant name, CHI number, and date of birth will be retained for the duration of study follow-up (lifelong) in order to allow data linkage to primary and secondary healthcare records. All other personal data will be stored for 5 years.

8.5 Disposal of Data

Research data will be archived after the end of the study and made available in de-identified format to satisfy data sharing requirements at the end of the study.

8.6 External Transfer of Data

Participants consent to the use of information about them to support other research in the future, and that the information may be shared anonymously with other researchers, whatever happens to the participant.

Data Transfer will be in accordance with General Data Protection Regulations, ICH GCP and SOPs that cascade from the practices defined by the Academic and Clinical Central Office for Research and Development (ACCORD) SOPs.

Delegated research staff will enter the data required by the protocol into the eCRF following training in the definitions and methods used in completing the eCRF.

On completion of data collection, the Investigator must certify that the data entered into the eCRF is complete and accurate.

Data verification and cleaning will be performed as per local procedures and detailed in the Data Management Plan.

8.7 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).

8.8 Data Breaches

Any data breaches will be reported to the University of Edinburgh (dpo@ed.ac.uk) and NHS Lothian (Lothian.DPO@nhslothian.scot.nhs.uk) 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

This is a discovery study and no comparable cohort in terms of phenotypical depth exists. Based upon data collected from our first 150 participants, we anticipate that 330 women with repeated measures (cohort 1) and a parallel cohort of 225 with cross-sectional measures (cohort 2) will allow us to identify significant differences, where they exist, in retinal vascular parameters and their trajectories between healthy pregnancies and those affected by pre-eclampsia or fetal growth restriction, allowing for pregnancy losses and losses to follow up. A larger sample size in cohort 1 is necessary to ensure we capture a sufficient number of women who subsequently go on to develop fetal growth restriction or pre-eclampsia, based on the known background prevalence of these complications.

9.2 PROPOSED ANALYSES

We will perform:

1. Analysis of the retinal vessels using the fundus images. Our software measures changes in the retina that might be imperceptible to, or missed, by a human grader. Automatic detection or segmentation of the vessels by the software is followed by semi-automatic classification of arterioles and venules. Measurements pertaining to the retinal vasculature including vessel caliber, branching complexity (via fractal dimension and alpha shapes), and tortuosity are then performed (16, 19-21). These metrics characterise the state or condition of the microvascular system visible in the retina as an indicator microvascular health elsewhere in the human body.
2. Analysis of the deeper lying choroidal vessels using Optical Coherence Tomography (OCT). Computerised delineation of the boundaries of this layer enable measurements of thickness and volume, while automatic segmentation of the darker appearing vessels therein enable quantification of a choroidal vascular index to characterise the tissues in greater detail (22).
3. Optical Coherence Tomography Angiography (OCTA) allows rapid visualisation of the retinal microcirculation (for multiple capillary plexi within the different chorioretinal layers), and its potential clinical use has been demonstrated in several retinal and systemic diseases (4-7). OCTA is well suited for revealing subtle microvascular remodelling processes. In previous work, we have developed an OCTA analysis framework for the segmentation, skeletonisation, and network analysis of different regions of interest (4, 5). This approach allows for deep vascular phenotyping and patient clustering according to outcomes.

We will define ‘normal ranges’ of the measures for future comparison to other cohorts, as well assess variability in these imaging-derived metrics, so that we can determine associated uncertainty and levels of precision. A key feature of the SPECTRALIS platform is its eye tracking technology that ensures repeat scans of individuals are accurately positioned to capture the same view of the retina at different

examinations. This assists our study of individualised trajectories of changes in the retina in healthy uncomplicated pregnancies as we evaluate which parameters show promise as biomarkers of vascular response to pregnancy. While previous research programmes have examined the association of parameters obtained from retinal imaging during pregnancy, only a single imaging modality was typically used at a time (e.g. fundus images or OCTA). Our proposed approach to study the microvasculature using multi-modal retinal imaging supplemented with custom software will maximize the information provided by these non-invasive techniques, producing a more complete examination of the retina than has previously been utilized in studies of pregnancy. This will deliver a suite of validated candidate retinal biomarkers.

We have developed a pipeline for multimodal data integration and predictive modelling of outcomes ready to apply to pregnancy data (23).

To avoid overfitting in our proof-of-concept cohort, we will rely on the previously described feature engineering approaches for the retinal imaging datasets. These features will be integrated with the remaining tabular data (e.g. demographics, bloods, ultrasound, cardiovascular metrics) and used for predictive modelling of outcome measures. Outcomes will be predicted leveraging the whole dataset (data acquired in weeks 12 or 20, and 36) but also restricted to data acquired in weeks 36 alone (both with and without data acquired at post-natal follow-up, in the case of long-term cardiovascular outcomes). For continuous outcomes, this will be approached as a regression task. For categorical outcomes, predictive modelling will only be attempted for sufficiently balanced problems. Training/test data splits will be performed with k-fold cross validation in the training set for parameter tuning. Linear/logistic regression with regularisation will be employed for initial feature selection and constructing explainable, parsimonious linear models. When correlations between measurements at different time points are discovered, a generalised estimating equation will be used to estimate the average response over the population rather than specific regression parameters. Finally, non-linear models such as gradient boosting will be evaluated due to the superior performance in some modelling scenarios (18, 23). Classifiers will be evaluated in terms of precision, recall, and area under the receiver-operator curve and compared with clinically meaningful baselines based on demographic, lifestyle, and routine clinical evaluation data.

10 RISKS

All researchers involved in this study envisage that the extent of adverse events would remain low. All measurements are non-invasive and have been conducted safely in pregnancy without any adverse events. However, in the event of an adverse event, the appropriate investigator will investigate the causality, seriousness and severity. The only identified risk is in data security (Table 1).

Table 1: Main risks to data security

Risk description	Security controls	Likelihood	Impact	Risk
Unauthorised access to patient records	NHSL contract of employment. Personnel mandatory training	Low	Medium	Low
Unauthorised access to NHSL servers	Role based and password protected access to NHSL systems with staff working on dedicated NHSL equipment is secure locations	Low	High	Medium

Risk of disclosure of data leaving the NHSL server	Data extracts pseudonymised, disclosure checked before release. SOPs and training according to national guidance.	Low	High	Medium

11 OVERSIGHT ARRANGEMENTS

11.1 INSPECTION OF RECORDS

Investigators and institutions involved in the study will permit study 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 a study specific risk assessment is required.

If required, a study specific risk assessment will be performed by representatives of the Sponsor(s), ACCORD monitors and the QA group, in accordance with ACCORD governance and sponsorship SOPs. Input will be sought from the Chief Investigator or designee. The outcomes of the risk assessment will form the basis of the monitoring plans and audit plans.

If considered necessary, ACCORD clinical trial monitors, or designees, will perform monitoring activities in accordance with the study monitoring plan. This will involve on-site visits and remote monitoring activities as necessary. ACCORD QA personnel, or designees, will perform study audits in accordance with the study audit plan. This will involve investigator site audits, study management audits and facility (including 3rd parties) audits as necessary (delete where not required).

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 necessary 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.

Delegated tasks must be documented on a Delegation Log and signed by all those named on the list prior to undertaking applicable study-related procedures.

12.2.1 Informed Consent

The Investigator is responsible for ensuring informed consent is obtained before any study 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 study team and the participant will sign and date the Informed Consent Form(s) to confirm that consent has been obtained. The original will be signed in the Investigator Site File (ISF). The participant will receive a copy of the signed consent form and a copy will be filed in the participant's medical notes.

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 study 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. 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 Data Protection Training

All University of Edinburgh employed researchers and study staff will complete the Data Protection Training through Learn.

NHS Lothian employed researchers and study staff will comply with NHS Lothian mandatory Information Governance Data Protection training through LearnPro.

Non-NHS Lothian staff that have access to NHS Lothian systems will familiarise themselves and abide by all NHS Lothian IT policies, as well as employer policies

12.2.7 Information Security Training

All University of Edinburgh employed researchers, students and study staff will complete the Information Security Essentials modules through Learn and will have read the minimum and required reading setting out ground rules to be complied with.

NHS Lothian employed researchers and study staff will comply with NHS Lothian mandatory Information Governance IT Security training through LearnPro.

Non-NHS Lothian staff that have access to NHS Lothian systems will familiarise themselves and abide by all NHS Lothian IT policies, as well as employer policies

12.2.8 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.

12.2.9 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 that could allow identification of individual participants.

13 STUDY CONDUCT RESPONSIBILITIES

13.1 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.

Proposed amendments will be submitted to the Sponsor for classification, review and authorisation.

Amendments to the protocol must be submitted in writing to the appropriate REC and local R&D for approval prior to implementation and prior to participants being enrolled into the amended protocol.

13.2 MANAGEMENT OF PROTOCOL NON COMPLIANCE

13.2.1 Protocol Waivers

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.

13.2.2 Management of Deviations and Violations

Deviations and violations are non-compliance events discovered after the event has occurred. 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.

Deviation logs will be maintained for each site in multi-centre studies.

Deviation logs/violation forms will be transmitted via email to QA@accord.scot. Only forms in a pdf format will be accepted by ACCORD via email. Forms may also be submitted by hand to the office. Where missing information has not been sent to ACCORD after an initial report, ACCORD will contact

the Investigator and request the missing information. The Investigator must respond to these requests in a timely manner.

13.3 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 study; or

(b) the scientific value of the study.

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

13.4 STUDY RECORD RETENTION

All study documentation will be kept for a minimum of 3 years from the protocol defined end of study point. When the minimum retention period has elapsed, study documentation will be destroyed with permission from the Sponsor.

13.5 END OF STUDY

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

The Investigators and/or the 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 Sponsor(s) 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 Sponsor(s) via email to researchgovernance@ed.ac.uk.

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

13.6 CONTINUATION OF TREATMENT FOLLOWING THE END OF STUDY

Not applicable.

13.7 INSURANCE AND INDEMNITY

The Sponsor(s) 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 Sponsor(s)' 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 Sponsor(s) 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.

14 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team.

15 REFERENCES

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