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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-030353
Article Type:	Protocol
Date Submitted by the Author:	24-Mar-2019
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Keywords:	Ultrasonography < OBSTETRICS, Prenatal diagnosis < OBSTETRICS, Ultrasound < RADIOLOGY & IMAGING, Physiology < BASIC SCIENCES, Pathology < BASIC SCIENCES

Assessment of uteroplacental vascularisation in early first-trimester pregnancy with contrast-enhanced ultrasound and 3D power Doppler angiography: a multicentre prospective study (HOPE).

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Abstract

Introduction: Knowledge about the mechanisms leading to the establishment of uteroplacental vascularisation is inadequate, and some of what has been thought to be known for decades has recently been challenged by a showing that the intervillous space, the major area of maternal-fetal exchange, appears to be perfused by maternal blood at as early as 6 weeks of gestation. The vascular flow then seems relatively constant until 13 weeks, when it appears to increase suddenly.

Objectives: The principal objective is to quantify the perfusion of the intervillous space by contrast-enhanced ultrasonography (CEUS) during the first-trimester at three different gestational ages (8, 11, and 13 weeks). The secondary objectives are to: (i) describe the indicators of vascularisation of the placenta (intervillous space) and the myometrium at the three gestational ages, measured by CEUS and 3D power Doppler (3DPD) angiography; (ii) compare the diagnostic performance of CEUS and 3DPD for the demonstration and quantification of uteroplacental vascularisation; and (iii) establish a biological collection of placentas to increase knowledge about placental development and functions during pregnancy.

Methods and Analysis: This is a prospective, cross-sectional, multicentre, non-randomised, open study. We will include 42 women with ongoing pregnancy and divided into three group of gestational ages (that is, 14 women by per group): 8, 11, and 13 weeks of gestation. 3DPD and then CEUS will be performed and the data about the perfusion kinetics and the 3DPD indices will be calculated and then compared with each other and for each gestational age.

Ethics: The appropriate French Ethics Committee (CPP, Comité de Protection des Personnes) Est III approved this study and the related consent forms on April 5, 2016, and the competent authority (ANSM, Agence Nationale de Sécurité du Médicament et des Produits de Santé) authorised the study on June 21, 2016.

Strengths and limitations of this study

- The recent data will be confirmed in our study with a larger number of women.
- To our knowledge, this will be the first study comparing 3DPD with CEUS. Should the results demonstrate the value of 3DPD, studies with a longitudinal follow-up, that is, of ongoing pregnancies, could be conducted and would enable imaging data to be correlated with final pregnancy outcomes.
- The groups are selected by gestational age.
- The pathophysiologic interpretation of the data will be limited by the absence of information about pregnancy outcome.

INTRODUCTION

Preeclampsia and intrauterine growth restriction (IUGR) are two principal complications of pregnancy that account for more than 30% of maternal and fetal morbidity and mortality. These diseases, which affect from 4 to 7% of pregnancies, are related to chronic uteroplacental hypoperfusion, and knowledge of its pathophysiologic mechanisms remains inadequate. Currently, the major hypothesis is that defective trophoblastic invasion during the first trimester leads to chronic uteroplacental hypoperfusion (1).

The mechanisms that establish uteroplacental vascularisation during the first trimester were described several decades ago, based on pathology studies, in the absence of functional imaging tools applicable *in vivo* in pregnant women(2,3). The presence of trophoblastic (also called endovascular) plugs in the spiral arteries was thought to prevent perfusion of the intervillous space until approximately 10 weeks of gestation, to enable the trophoblast to develop in a favourable hypoxic situation. More recently, ultrasound exploration seemingly confirming the absence of a Doppler signal before 12 weeks strengthened this hypothesis(4,5). Other work, notably histologic exploration of early-pregnancy hysterectomy specimens suggesting the absence of vascular "connection" before 8 weeks of gestation between the maternal network and the intervillous space and physiological explorations showing the maintenance of a hypoxic environment within the placenta up to 10 weeks of gestation also pointed in this direction at the beginning of this century (2,6).

On the other hand, the chronology and conditions leading to the disappearance of these plugs remained unknown. Based on this knowledge, it was suggested the origin of the chronic placental hypoperfusion phenomena observed in preeclampsia and intrauterine growth restriction might be the premature disappearance of these plugs and therefore the loss of the hypoxic environment (7–11).

This entire set of pathophysiologic hypotheses was called into question in 2017. Specifically, Roberts et al. (*Hum Reprod*, 2017) applied a modern technique of functional imaging — contrast-enhanced ultrasonography (CEUS) — to the placenta to show that the intervillous space was perfused by 6 weeks of gestation(12).They suggested in particular that the plugs disappear between 6 and 8 weeks.

This exploratory work thus presents major interest in terms of the physiological understanding of uteroplacental perfusion in the first trimester. Nonetheless, the study design and the number of pregnancies studied did not make it possible to go farther in understanding the quantitative course of this perfusion of the intervillous space. Accordingly, the authors observed quantitative modification of vascular flow within the intervillous space only starting at 13 weeks; they accordingly hypothesised that late remodelling of the radial arteries led to the reduction of vascular resistance. It is nonetheless also possible that the study lacked the power to show quantitative differences in vascular flow between 6 and 13 weeks.

These new data must imperatively be confirmed by other studies with more subjects, designed in principle with the objective of quantifying flow as a function of gestational age. The

demonstration of blood within the intervillous space at 6 weeks of gestation was possible through the use of an ultrasound contrast product, known to be strictly intravascular.

3D power Doppler (3DPD) angiography is another innovative technique for functional placental imaging. First described in 2004, it has the major interest of being usable in pregnant women because it has no teratogenic risk(13). It appears to present a major potential interest for the study of pathophysiologic phenomena associated with uteroplacental vascularisation(14,15). This technique, however, has only been assessed at a gestational age of 11 weeks (16). Its principal limitations are the absence of absolute specificity of the Doppler signal for blood flow and the impossibility of differentiating maternal and fetal flow. Accordingly, it would be interesting to assess whether 3DPD angiography might make it possible to quantify early perfusion of the intervillous space, by comparing it with CEUS, which is specific for maternal blood flow. If this is the case, contrast product would no longer be necessary for exploring uteroplacental vascularisation during pregnancy.

OBJECTIVE

Primary objective

The principal objective is to quantify the perfusion of the intervillous space by CEUS during the first trimester at three different gestational ages: 8, 11, and 13 weeks.

Secondary objectives

- Describe the indicators of vascularisation of the placenta (intervillous space) and the myometrium at the three gestational ages, measured by CEUS and 3DPD.

- Compare the diagnostic performance of CEUS and 3DPD for the demonstration and quantification of uteroplacental vascularisation.

- Establish a biological collection of placentas to increase knowledge about placental development and functions during pregnancy.

METHODS

Trial design

Prospective, physiological, cross-sectional, multicentre, and non-randomised open study. This is a multicentre study including a level III university hospital centre (CHRU Nancy) and a level II regional hospital centre (CHR Metz).

Study population

Table 1 presents the inclusion and non inclusion criteria and Figure 1 presents the flow chart of the study.

The participants in this study are women with ongoing pregnancy at the first-trimester. The analysis of uteroplacental vascularisation by CEUS can only be performed in contexts of elective abortion, as the contrast product is not authorised for use in ongoing pregnancies.

Before inclusion, the women will be informed of the aim, the procedures, and the predictable risks of the study (no identified clinical risk) by the principal investigator.

US acquisition

The ultrasound acquisition will begin in 2D mode by measurement of the crown-rump length to verify gestational age and study group. The position of the placenta will be noted and the Doppler spectrum of the uterine arteries, spiral arteries, and radial arteries will be recorded, with measurements of the pulsatility and resistance indices.

First, the 3D Doppler acquisitions will be performed at 4 different predetermined settings (impact of the settings currently underway: NCT03342014). The instrument used will be a Voluson S8 (General Electric Healthcare) with a volumetric convex abdominal transducer (4–8 MHz).

Next, the CEUS acquisitions will be performed with a Logiq E9 (General Electric Healthcare) and an abdominal transducer (1-5 MHz). The ultrasound contrast product used will be Sonovue® (Bracco Imaging, Italy), administered by bolus injection. A volume of 2.4 mL of contrast product will be injected per patient, and repeated once if necessary.

Image analysis

3DPD angiography: The images will be analysed with VOCAL® software, which makes it possible to define a volume of interest and to quantify the Doppler signals to calculate the vascularisation indices (VI, FI, and VFI) automatically (Figure 2). Each volume will be recorded and analysed independently. Two regions of interest will be traced: one focused on the placenta and the other on the myometrium (placental bed).

CEUS: The images will be analysed with specific software that makes it possible to trace the regions of interest and to view the perfusion curves and to extract semiquantitative perfusion indicators from them (Figure 3).

Placenta collection and analyses

The placentas will be collected and transferred fresh for preparation. The placental villi will be isolated and rinsed in saline solution. A portion of the villi (approximately 1 to 2 cm^3 of tissue) will be frozen at -80°C for the subsequent extraction of RNA and specific proteins. For an immunohistochemical analysis, a second portion will be fixed in buffered formalin for inclusion in paraffin and serial sections of 5-µm will be made on superfrost slides. The markers of oxidative stress will be specifically studied.

Outcomes

The principal endpoint is the measurement (in arbitrary units) of signal intensity in the intervillous space during the first trimester (at 8, 11, and 13 weeks), obtained by CEUS.

The secondary outcome measures are:

1. Measurement of indicators of vascularisation in the intervillous space and the myometrium: signal intensity and perfusion kinetics.
2. Comparison of the quantitative data about uteroplacental vascularisation obtained with each technique.
3. Procurement of placental villi that can be analysed to study human placental development and functions.

Participant timeline

The enrolment of women began in February 2019. In view of the recruitment capacity of our institutions, the recruitment should be completed by November 2019.

Patient and public involvement

Patients and public were not involved.

Premature ending of patient participation

Participants will be excluded from the study in the following situations:

- Lack of CEUS acquisition
- Withdrawal of consent before the end of the study.

Patients will be immediately excluded from the study and replaced with other new participants. Any decision to withdraw consent will not affect the patient's routine medical care. In the case of an adverse event related to the study, the patient will be informed and excluded. She will also receive what additional medical care is necessary or appropriate.

Follow-up

No specific follow-up has been planned for participants except for standard routine healthcare. Any adverse events will be noted and reported.

Sample size consideration

In view of recent data from the literature (Roberts, *Hum Reprod*. 2017), to show a difference in signal intensity between the three groups (8, 11, and 13 weeks) with α (corrected for the multiple comparison) and β risks set respectively at 0.017 and 10%, we should have 14 women in each group at analysis, that is, 42 women in all.

Data collection and management:

An electronic case report file (e-CRF) will be created for each woman. The women's anonymity will be ensured by mentioning to the maximum extent possible their research code number,

followed by the first letters of the last name and first name of the participant on all necessary documents or by deleting their names by appropriate means (white-out) from the copies of source documents intended to document the study.

The ultrasound data will be anonymised and transferred via a secure server for storage and archiving directly in the ARCHIMED database, reported to the CNIL (CNIL report number: 1410005) because they cannot be transcribed in the e-CRF. The clinical data concerning the woman collected are in Table 2.

Statistical analysis

The quantitative indicators will be described by their means ± standard deviations, medians, and maximum and minimum values, the qualitative indicators by the number of individuals and percentages. The mean values will be compared between the groups by Student's t test or the Mann-Whitney test, matched or not, depending on the type of data. The comparison of imaging techniques will be completed by Bland-Altman plots.

The analyses will be performed with R software.

No interim analysis is planned.

No statistical criterion for stopping the study is planned.

QUALITY CONTROL

Right of access to data and source documents

The medical data of each patient will only be transmitted to the sponsor (Metz-Thionville Regional Hospital Centre, CHR Metz-Thionville) or any person duly authorised by the sponsor and, where applicable, to the authorised health authorities, under conditions guaranteeing their confidentiality.

The sponsor and the regulatory authorities may request direct access to the medical file for verification of the procedures and/or data of the clinical trial, without violating confidentiality and within the limits permitted by laws and regulations.

For research purposes, the processing of personal data relating to persons undergoing research will be carried out.

These data are collected and processed solely on the basis of the legal grounds provided for by statute and regulations in the context of the performance of the public interest missions of the Metz-Thionville CHR, in particular those relating to ensuring and contributing to research and innovation (Article 6.1.e of the GDPR, General Data Protection Regulation). The processing of personal data of persons participating in research is permitted by the exception provided for in Article 9.2(i) and (j).

This data processing is part of the MR001 reference methodology that the Metz-Thionville CHR has undertaken to respect.

In accordance with the GDPR, persons participating in research have a right of access to their data (Article 15), a right of rectification (Article 16), a right to erase their data (right to forget) under the conditions provided for in Article 17, a right to limit the processing provided for in Article 18, and a right to object to the processing of their personal data (Article 21). These rights are exercised with the Investigators, who will inform the research sponsor as soon as possible.

The persons participating in the research also have a right to complain to the supervisory authority in France, namely, the Commission Nationale de l'Informatique et des Libertés (CNIL).

Study monitoring

The monitoring will be performed by the appropriate department of the Support Platform for Clinical Research (PARC) of CHR Metz-Thionville, throughout the study.

A Clinical Research Associate (CRA) will travel regularly to each centre to perform the quality control of the e-CRF data.

The CRA will verify that the research is conducted according to the protocol provided in accordance with regulations and will ensure that every e-CRF contains all of the information requested.

Each patient's e-CRF must be consistent with the source documents.

The investigators will allocate adequate time for such monitoring activities. The investigators will also make sure that the monitor or other compliance or quality assurance reviewer is given access to all of the above noted study-related documents and study-related facilities (e.g. Ultrasound, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visits.

Data management and quality control

Data management will be carried out by the clinical research team CIC-IT of Nancy (INSERM CIC 1433). The data images (ultrasound) will be automatically transferred to Nancy CIC-IT and stored after verification in the ArchiMed database declared to the French authorities (CNIL declaration number: 1410005).

Patient data protection

Each patient must be identified on the e-CRF, ultrasound data, and placenta collection with her initials and identification number indicating her order of inclusion into the study. The investigators must keep the list of all the patients, including identification numbers, full names and last known addresses.

Patients must be informed in writing about the possibility of audits by authorised representatives of the sponsor and/or regulatory authorities, in which case the relevant parts of study-related hospital records may be required.

Patients must also be informed that the results obtained will be computerised and analysed, that local laws will be applied, that their confidentiality will be preserved, and that they are entitled to obtain any information concerning the data stored and analysed by the computerised system.

POTENTIAL RISKS TELATED TO THE STUDY

This study may expose the women participating in it to rare, transient, mild side effects. It will comply at all times with the Good Clinical Practices defined by the Ministry of Health.

The only constraint associated with the study is the addition of 3D Doppler acquisitions after the standard 2D ultrasound; these acquisitions will prolong the procedure by 10 minutes. The medical devices (Voluson® and Logiq E9®, GE Healthcare) are CE-marked and used routinely in clinical practice.

The contrast product (SonoVue®) is authorised for use in exploring lesions of the liver, breasts, and great vessels. It will be used according to the guidelines described in the summary of product characteristics in its French marketing authorisation. In the current state of knowledge about the safety of contrast products (which have not yet been authorised for use during pregnancy), adequate evidence exists to affirm their absence of permanent or serious adverse effects among women.

ETHICAL PERMISSION

The sponsor and all investigators undertake to conduct this study in accordance with the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects, Tokyo 2004) and its updates, the provisions of European Directive 2001/20-CE as transposed into French law by L. 2004-806 dated August 9, 2004, on public health policy and 2004-800 dated August 6, 2004, on bioethics and their implementation decrees, and to comply with the guidelines of Good Clinical Practices (I.C.H. version 4 of May 1, 1996 and Decision of November 24, 2006).

They undertake to adhere to all legislative and regulatory provisions that may concern the research. In accordance with Article L. 1123-6 of the Public Health Code, the sponsor has submitted the research protocol the sponsor to the appropriate Committee for the Protection of Persons (CPP, Patient Protection Committee) Est III, which approved this study and the related consent forms on April 5, 2018 (Number 16.03.02). The competent authority (ANSM, Agence Nationale de Sécurité du Médicament et des Produits de Santé) authorised the study on June 21, 2018 (Number 160187A 12).

The study is registered under the number NCT02884297 on clinicaltrial.gov and under the number 2015-005655-27 on EudraCT.

The research is to be conducted in accordance with the present Protocol. The Investigators undertake to respect the protocol in all respects especially with regard to obtaining consent and the notification and follow-up of serious adverse events.

Information letter and informed consent

Research participants will be informed of the objectives and constraints of the study, their rights to refuse to participate in the study, or to withdraw from the study at any time. When all essential information has been conveyed to the subject and the investigators have ensured that the patient has understood the implications of participating in the trial, the patient's written consent shall be obtained by an investigator in two original copies. A copy of the information forms and signed consents will be given to the patient.

The investigator will retain the second copy for a minimum of 15 years.

PROTOCOL AMENDMENT

The sponsor must be informed of any proposed amendment to the protocol by the coordinating investigator. The changes must be described, substantive or not.

A substantial change is a change which is susceptible, in one way or another, to modify the assurances made to participants who consent to biomedical research (modification of an inclusion criterion, extending the inclusion period, participation of new centre, etc.).

Once the research has begun, any substantial modification thereof at the initiative of the sponsor must obtain, prior to its implementation, a favourable approval of the committee and an authorisation from the competent authority. In this case, if necessary, the committee ensures that a new consent from individuals participating in research is obtained.

Any substantial change requires that an authorisation request be made by the sponsor to the ANSM and/or a notification request by the CPP in accordance with legislative regulation n ° 2004-806 of August 9, 2004.

FINAL RESEARCH REPORT

The coordinator and the mandated biostatistician will collaboratively write the final research report. This report will be submitted to each of the investigators for review. Once consensus has been reached, the final version must be endorsed with the signature of each of the investigators and sent to the sponsor as early as possible after the effective end of the research. A report prepared according to the reference plan of the competent authority must be forwarded to the competent authority and the CPP within a year after the end of the research.

DISCUSSION

This study is a follow-up to that recently published by Roberts et al.(12)

Our first objective is to confirm their data, that is, the existence of early perfusion of the intervillous space (from 8 weeks of gestation) and an increase in vascular flow starting at 13 weeks. We also seek to explore the course of vascular flow rates with gestational age to test both of the hypotheses advanced about the absence of modification of flow before 13 weeks: lack of power or external regulatory factors (remodelling of the radial arteries)?

To test the first hypothesis, we chose to increase the number of individuals in each group and to select three gestational age targets: 8, 11, and 13 weeks of gestation. A significant result

between 8 and 13 weeks and between 11 and 13 weeks is expected and would thus confirm the data of Roberts et al. The intermediate group at 11 weeks was chosen arbitrarily and its size was calculated to be able to show a difference between 8 and 11 weeks.

To test the second hypothesis, and as Roberts & Frias have suggested, we will examine vascular resistance of the radial arteries at these different gestational ages and assess the level of correlation between the changes in resistance and in blood flow within the intervillous space (5,17,18).

The major difference between their study and ours is the choice of contrast product, a choice linked solely to the product available by geographic zone. They used Definity® while in Europe, we use Sonovue®. In principle this difference will have no effect on the comparison of our results, as their properties and performance are similar (19).

Moreover, we have chosen to perform 3DPD angiography at the same time to assess the applicability of this technique for the detection of blood flow before 11 weeks. Should there be a high correlation between the 3DPD indices and the indicators of perfusion by CEUS, future studies will be able to choose only the 3DPD, which will make it possible to envision a longitudinal follow-up.

Contributors: C.B. participated in study design and is carrying out or will carry out recruitment, Ultrasound acquisition, placental analysis, data analysis and manuscript writing. M.T. is carrying out or will carry out recruitment, ultrasound acquisition and data analysis. ML.E. and O.M. are major investigators for clinical assessment, Ultrasound acquisition and study design. AC and NM are project managers. GH is in charge of statistical analysis. All authors read and approved the final manuscript.

Fundings: This study won the regional award for clinical research (2016).

Competing interests: None declared

Patient consent: Obtained

Ethics approval: ANSM (the French National Agency for Medicines and Health Products Safety) and the Committee for the Protection of Persons (Comité de Protection des Personnes, CPP) approved this study.

Data sharing statement : All data generated during the project will be made freely available via CIC-IT, Nancy. Data obtained from this study will be deposited at CIC-IT Nancy where they will be maintained for a minimum of 15 years. There are no security, licensing or ethical issues related to the expected data, and all data used in the project will be generated directly as a result of the project, without any pre-existing data being used.

Trial status : This is an ongoing trial. Recruitment began in February 2019. We expect to complete recruitment by November 2019. We plan to publish final results in 2020.

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Tables and Figures

Table 1 : Inclusion and non inclusion criteria

Table 2 : Data collected

Figure 1 : Flow Chart

Figure 2 : Three dimensional Power Doppler (3DPD) Analysis

Figure 3 : Contrast-enhanced Ultrasound (CEUS) Analysis

Table 1 : Inclusion and non inclusion criteria

Inclusion criteria	Non inclusion criteria
- Woman age ≥ 18 years - Gestational age **8 WG Group :** 8 to 8+6 **11 WG Group :** 11 to 11+6 **13 WG Group :** 13 to 13+6 - Body Mass Index ≤ 40 kg/m² - Have given informed and written consent - Have confirmed her request of surgical elective abortion	- Contraindication to contrast injection : Hypersensitivity to sulfur hexafluoride or any of the other ingredients, history of cardiac disease, respiratory distress syndrome, severe pulmonary hypertension - Woman considered at risk of IUGR/PE : History of PE or IUGR, Autoimmune disease, Chronic High Blood Pressure, Diabetes - Woman unable to understand or follow the procedure of the study

Table 2 : Data collected

Family History	Preeclampsia, intrauterine growth restriction, venous thromboembolic disease, high blood pressure, diabetes
Obstetrical History	Parity, history of post-partum hemorrhage, history of pregnancy loss or intrauterine fetal death Fibroma or uterine malformation
Clinical data	Body Mass Index Proteinuria Blood pressure (systolic and diastolic) Smoking
Current treatments	

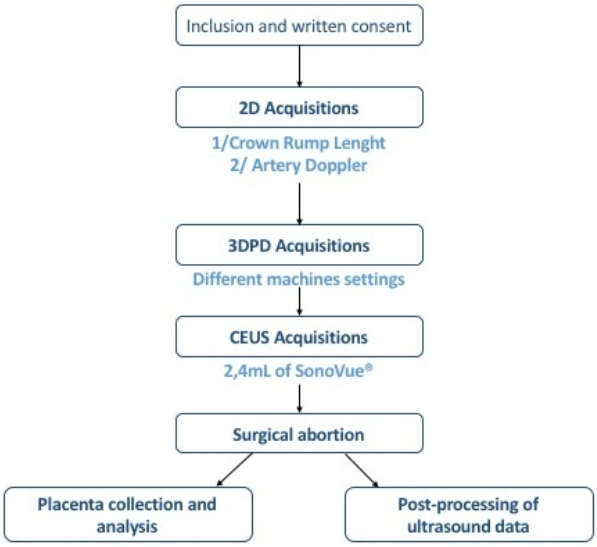

Figure 1: Flow chart

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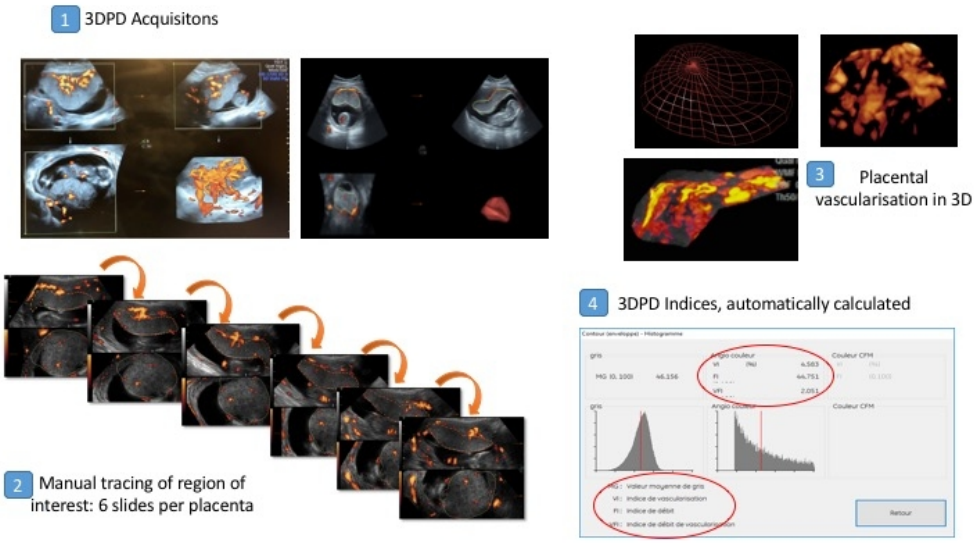

Figure 2: Three dimensional Power Doppler (3DPD) Analysis

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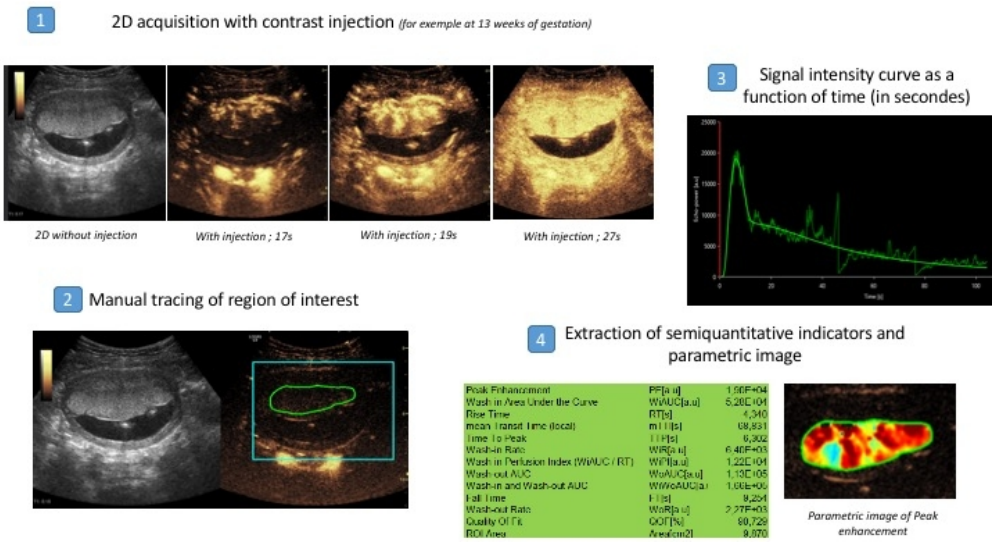

Figure 3: Contrast-enhanced Ultrasound (CEUS) Analysis

338x190mm (54 x 54 DPI)

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-030353.R1
Article Type:	Protocol
Date Submitted by the Author:	13-Jun-2019
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Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Radiology and imaging
Keywords:	Ultrasonography < OBSTETRICS, Prenatal diagnosis < OBSTETRICS, Ultrasound < RADIOLOGY & IMAGING, Physiology < BASIC SCIENCES, Pathology < BASIC SCIENCES

Assessment of uteroplacental vascularisation in early first-trimester pregnancy with contrast-enhanced ultrasound and 3D power Doppler angiography: protocol for a multicentre prospective study (HOPE).

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Abstract

Introduction: Knowledge about the mechanisms leading to the establishment of uteroplacental vascularisation is inadequate, and some of what has been thought to be known for decades has recently been challenged by a showing that the intervillous space, the major area of maternal-fetal exchange, appears to be perfused by maternal blood at as early as 6 weeks of gestation. The vascular flow then seems relatively constant until 13 weeks, when it appears to increase suddenly.

Objectives: The principal objective is to quantify the perfusion of the intervillous space by contrast-enhanced ultrasonography (CEUS) during the first-trimester at three different gestational ages (8, 11, and 13 weeks). The secondary objectives are to: (i) describe the indicators of vascularisation of the placenta (intervillous space) and the myometrium at the three gestational ages, measured by CEUS and 3D power Doppler (3DPD) angiography; (ii) compare the diagnostic performance of CEUS and 3DPD for the demonstration and quantification of uteroplacental vascularisation; and (iii) establish a biological collection of placentas to increase knowledge about placental development and functions during pregnancy.

Methods and Analysis: This is a prospective, cross-sectional, multicentre, non-randomised, open study. We will include 42 women with ongoing pregnancy and divided into three group of gestational ages (that is, 14 women by per group): 8, 11, and 13 weeks of gestation. 3DPD and then CEUS will be performed and the data about the perfusion kinetics and the 3DPD indices will be calculated and then compared with each other and for each gestational age.

Ethics and Dissemination: The appropriate French Ethics Committee Est III approved this study and the related consent forms on April 5, 2016, and the competent authority (ANSM) authorised the study on June 21, 2016. The results of this study will be published in a peer-reviewed journal and will be presented at relevant conferences.

Strengths and limitations of this study

- The recent data will be confirmed in our study with a larger number of women.
- To our knowledge, this will be the first study comparing 3DPD with CEUS. Should the results demonstrate the value of 3DPD, studies with a longitudinal follow-up, that is, of ongoing pregnancies, could be conducted and would enable imaging data to be correlated with final pregnancy outcomes.
- The groups are selected by gestational age.
- The pathophysiologic interpretation of the data will be limited by the absence of information about pregnancy outcome.

INTRODUCTION

Preeclampsia and intrauterine growth restriction (IUGR) are two principal complications of pregnancy that account for more than 30% of maternal and fetal morbidity and mortality. These diseases, which affect from 4 to 7% of pregnancies, are related to chronic uteroplacental hypoperfusion, and knowledge of its pathophysiologic mechanisms remains inadequate. Currently, the major hypothesis is that defective trophoblastic invasion during the first trimester leads to chronic uteroplacental hypoperfusion (1).

The mechanisms that establish uteroplacental vascularisation during the first trimester were described several decades ago, based on pathology studies, in the absence of functional imaging tools applicable *in vivo* in pregnant women(2,3). The presence of trophoblastic (also called endovascular) plugs in the spiral arteries was thought to prevent perfusion of the intervillous space until approximately 10 weeks of gestation, to enable the trophoblast to develop in a favourable hypoxic situation. More recently, ultrasound exploration seemingly confirming the absence of a Doppler signal before 12 weeks strengthened this hypothesis(4,5). Other work, notably histologic exploration of early-pregnancy hysterectomy specimens suggesting the absence of vascular "connection" before 8 weeks of gestation between the maternal network and the intervillous space and physiological explorations showing the maintenance of a hypoxic environment within the placenta up to 10 weeks of gestation also pointed in this direction at the beginning of this century (2,6).

On the other hand, the chronology and conditions leading to the disappearance of these plugs remained unknown. Based on this knowledge, it was suggested the origin of the chronic placental hypoperfusion phenomena observed in preeclampsia and intrauterine growth restriction might be the premature disappearance of these plugs and therefore the loss of the hypoxic environment (7–11).

This entire set of pathophysiologic hypotheses was called into question in 2017. Specifically, Roberts et al. (*Hum Reprod*, 2017) applied a modern technique of functional imaging — contrast-enhanced ultrasonography (CEUS) — to the placenta to show that the intervillous space was perfused by 6 weeks of gestation(12).They suggested in particular that the plugs disappear between 6 and 8 weeks.

This exploratory work thus presents major interest in terms of the physiological understanding of uteroplacental perfusion in the first trimester. Nonetheless, the study design and the number of pregnancies studied did not make it possible to go farther in understanding the quantitative course of this perfusion of the intervillous space. Accordingly, the authors observed quantitative modification of vascular flow within the intervillous space only starting at 13 weeks; they accordingly hypothesised that late remodelling of the radial arteries led to the reduction of vascular resistance. It is nonetheless also possible that the study lacked the power to show quantitative differences in vascular flow between 6 and 13 weeks.

These new data must imperatively be confirmed by other studies with more subjects, designed in principle with the objective of quantifying flow as a function of gestational age. The

demonstration of blood within the intervillous space at 6 weeks of gestation was possible through the use of an ultrasound contrast product, known to be strictly intravascular.

3D power Doppler (3DPD) angiography is another innovative technique for functional placental imaging. First described in 2004, it has the major interest of being usable in pregnant women because it has no teratogenic risk(13). It appears to present a major potential interest for the study of pathophysiologic phenomena associated with uteroplacental vascularisation(14,15). This technique, however, has only been assessed at a gestational age of 11 weeks (16). Its principal limitations are the absence of absolute specificity of the Doppler signal for blood flow and the impossibility of differentiating maternal and fetal flow. Accordingly, it would be interesting to assess whether 3DPD angiography might make it possible to quantify early perfusion of the intervillous space, by comparing it with CEUS, which is specific for maternal blood flow. If this is the case, contrast product would no longer be necessary for exploring uteroplacental vascularisation during pregnancy.

OBJECTIVE

Primary objective

The principal objective is to quantify the perfusion of the intervillous space by CEUS during the first trimester at three different gestational ages: 8, 11, and 13 weeks.

Secondary objectives

- Describe the indicators of vascularisation of the placenta (intervillous space) and the myometrium at the three gestational ages, measured by CEUS and 3DPD.

- Compare the diagnostic performance of CEUS and 3DPD for the demonstration and quantification of uteroplacental vascularisation.

- Establish a biological collection of placentas to increase knowledge about placental development and functions during pregnancy.

METHODS

Trial design

Prospective, physiological, cross-sectional, multicentre, and non-randomised open study. This is a multicentre study including a level III university hospital centre (CHRU Nancy) and a level II regional hospital centre (CHR Metz).

Study population

Table 1 presents the inclusion and non inclusion criteria and Figure 1 presents the flow chart of the study.

The participants in this study are women with ongoing pregnancy at the first-trimester. The analysis of uteroplacental vascularisation by CEUS can only be performed in contexts of elective abortion, as the contrast product is not authorised for use in ongoing pregnancies.

Before inclusion, the women will be informed of the aim, the procedures, and the predictable risks of the study (no identified clinical risk) by the principal investigator.

US acquisition

The ultrasound acquisition will begin in 2D mode by measurement of the crown-rump length to verify gestational age and study group. The position of the placenta will be noted and the Doppler spectrum of the uterine arteries, spiral arteries, and radial arteries will be recorded, with measurements of the pulsatility and resistance indices.

First, the 3D Doppler acquisitions will be performed at 4 different predetermined settings (impact of the settings currently underway: NCT03342014). The instrument used will be a Voluson S8 (General Electric Healthcare) with a volumetric convex abdominal transducer (4–8 MHz).

Next, the CEUS acquisitions will be performed with a Logiq E9 (General Electric Healthcare) and an abdominal transducer (1-5 MHz). The ultrasound contrast product used will be Sonovue® (Bracco Imaging, Italy), administered by bolus injection. A volume of 2.4 mL of contrast product will be injected per patient, and repeated once if necessary.

Settings and equipments are standardized between the two centers.

Image analysis

3DPD angiography: The images will be analysed with VOCAL® software, which makes it possible to define a volume of interest and to quantify the Doppler signals to calculate the vascularisation indices (VI, FI, and VFI) automatically (Figure 2). Each volume will be recorded and analysed independently. Two regions of interest will be traced: one focused on the placenta and the other on the myometrium (placental bed).

CEUS: The images will be analysed with specific software that makes it possible to trace the regions of interest and to view the perfusion curves and to extract semiquantitative perfusion indicators from them (Figure 3).

Placenta collection and analyses

The placentas will be collected and transferred fresh for preparation. The placental villi will be isolated and rinsed in saline solution. A portion of the villi (approximately 1 to 2 cm^3 of tissue) will be frozen at -80°C for the subsequent extraction of RNA and specific proteins. For an immunohistochemical analysis, a second portion will be fixed in buffered formalin for inclusion in paraffin and serial sections of 5-µm will be made on superfrost slides. The markers of oxidative stress will be specifically studied. The third portion will be fixed in formalin, diluted à 4%, and kept at 4°C in order to perform a confocal microscopic analysis.

All the villi will be collected and we consider that it is representative sampling of the placenta. However, it will be not possible to perform a spatial localization of the villi because placenta if surgically collected and as multiples parts.

Outcomes

The principal endpoint is the measurement (in arbitrary units) of signal intensity in the intervillous space during the first trimester (at 8, 11, and 13 weeks), obtained by CEUS.

The secondary outcome measures are:

1. Measurement of indicators of vascularisation in the intervillous space and the myometrium: signal intensity and perfusion kinetics.
2. Comparison of the quantitative data about uteroplacental vascularisation obtained with each technique.
3. Procurement of placental villi that can be analysed to study human placental development and functions.

Participant timeline

The enrolment of women began in October 2016. In view of the recruitment capacity of our institutions, the recruitment should be completed by November 2019.

Patient and public involvement

Patients and public were not involved.

Premature ending of patient participation

Participants will be excluded from the study in the following situations:

- Lack of CEUS acquisition
- Withdrawal of consent before the end of the study.

Patients will be immediately excluded from the study and replaced with other new participants. Any decision to withdraw consent will not affect the patient's routine medical care. In the case of an adverse event related to the study, the patient will be informed and excluded. She will also receive what additional medical care is necessary or appropriate.

Follow-up

No specific follow-up has been planned for participants except for standard routine healthcare. Any adverse events will be noted and reported.

Sample size consideration

In view of recent data from the literature (Roberts, *Hum Reprod*. 2017), to show a difference in signal intensity between the three groups (8, 11, and 13 weeks) with α (corrected for the

multiple comparison) and β risks set respectively at 0.017 and 10%, we should have 14 women in each group at analysis, that is, 42 women in all.

Data collection and management:

An electronic case report file (e-CRF) will be created for each woman. The women's anonymity will be ensured by mentioning to the maximum extent possible their research code number, followed by the first letters of the last name and first name of the participant on all necessary documents or by deleting their names by appropriate means (white-out) from the copies of source documents intended to document the study.

The ultrasound data will be anonymised and transferred via a secure server for storage and archiving directly in the ARCHIMED database, reported to the CNIL (CNIL report number: 1410005) because they cannot be transcribed in the e-CRF. The clinical data concerning the woman collected are in Table 2.

Statistical analysis

The quantitative indicators will be described by their means ± standard deviations, medians, and maximum and minimum values, the qualitative indicators by the number of individuals and percentages. The mean values will be compared between the groups by Student's t test or the Mann-Whitney test, matched or not, depending on the type of data. The comparison of imaging techniques will be completed by Bland-Altman plots.

The analyses will be performed with R software.

No interim analysis is planned.

No statistical criterion for stopping the study is planned.

QUALITY CONTROL

Right of access to data and source documents

The medical data of each patient will only be transmitted to the sponsor (Metz-Thionville Regional Hospital Centre, CHR Metz-Thionville) or any person duly authorised by the sponsor and, where applicable, to the authorised health authorities, under conditions guaranteeing their confidentiality.

The sponsor and the regulatory authorities may request direct access to the medical file for verification of the procedures and/or data of the clinical trial, without violating confidentiality and within the limits permitted by laws and regulations.

For research purposes, the processing of personal data relating to persons undergoing research will be carried out.

These data are collected and processed solely on the basis of the legal grounds provided for by statute and regulations in the context of the performance of the public interest missions of the Metz-Thionville CHR, in particular those relating to ensuring and contributing to research

and innovation (Article 6.1.e of the GDPR, General Data Protection Regulation). The processing of personal data of persons participating in research is permitted by the exception provided for in Article 9.2(i) and (j).

This data processing is part of the MR001 reference methodology that the Metz-Thionville CHR has undertaken to respect.

In accordance with the GDPR, persons participating in research have a right of access to their data (Article 15), a right of rectification (Article 16), a right to erase their data (right to forget) under the conditions provided for in Article 17, a right to limit the processing provided for in Article 18, and a right to object to the processing of their personal data (Article 21). These rights are exercised with the Investigators, who will inform the research sponsor as soon as possible.

The persons participating in the research also have a right to complain to the supervisory authority in France, namely, the Commission Nationale de l'Informatique et des Libertés (CNIL).

Study monitoring

The monitoring will be performed by the appropriate department of the Support Platform for Clinical Research (PARC) of CHR Metz-Thionville, throughout the study.

A Clinical Research Associate (CRA) will travel regularly to each centre to perform the quality control of the e-CRF data.

The CRA will verify that the research is conducted according to the protocol provided in accordance with regulations and will ensure that every e-CRF contains all of the information requested.

Each patient's e-CRF must be consistent with the source documents.

The investigators will allocate adequate time for such monitoring activities. The investigators will also make sure that the monitor or other compliance or quality assurance reviewer is given access to all of the above noted study-related documents and study-related facilities (e.g. Ultrasound, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visits.

Data management and quality control

Data management will be carried out by the clinical research team CIC-IT of Nancy (INSERM CIC 1433). The data images (ultrasound) will be automatically transferred to Nancy CIC-IT and stored after verification in the ArchiMed database declared to the French authorities (CNIL declaration number: 1410005).

Patient data protection

Each patient must be identified on the e-CRF, ultrasound data, and placenta collection with her initials and identification number indicating her order of inclusion into the study. The

investigators must keep the list of all the patients, including identification numbers, full names and last known addresses.

Patients must be informed in writing about the possibility of audits by authorised representatives of the sponsor and/or regulatory authorities, in which case the relevant parts of study-related hospital records may be required.

Patients must also be informed that the results obtained will be computerised and analysed, that local laws will be applied, that their confidentiality will be preserved, and that they are entitled to obtain any information concerning the data stored and analysed by the computerised system.

POTENTIAL RISKS RELATED TO THE STUDY

This study may expose the women participating in it to rare, transient, mild side effects. It will comply at all times with the Good Clinical Practices defined by the Ministry of Health.

The only constraint associated with the study is the addition of 3D Doppler acquisitions after the standard 2D ultrasound; these acquisitions will prolong the procedure by 10 minutes. The medical devices (Voluson® and Logiq E9®, GE Healthcare) are CE-marked and used routinely in clinical practice.

The contrast product (SonoVue®) is authorised for use in exploring lesions of the liver, breasts, and great vessels. It will be used according to the guidelines described in the summary of product characteristics in its French marketing authorisation. In the current state of knowledge about the safety of contrast products (which have not yet been authorised for use during pregnancy), adequate evidence exists to affirm their absence of permanent or serious adverse effects among women.

ETHICAL PERMISSION

The sponsor and all investigators undertake to conduct this study in accordance with the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects, Tokyo 2004) and its updates, the provisions of European Directive 2001/20-CE as transposed into French law by L. 2004-806 dated August 9, 2004, on public health policy and 2004-800 dated August 6, 2004, on bioethics and their implementation decrees, and to comply with the guidelines of Good Clinical Practices (I.C.H. version 4 of May 1, 1996 and Decision of November 24, 2006).

They undertake to adhere to all legislative and regulatory provisions that may concern the research. In accordance with Article L. 1123-6 of the Public Health Code, the sponsor has submitted the research protocol the sponsor to the appropriate Committee for the Protection of Persons (CPP, Patient Protection Committee) Est III, which approved this study and the related consent forms on April 5, 2018 (Number 16.03.02). The competent authority (ANSM, Agence Nationale de Sécurité du Médicament et des Produits de Santé) authorised the study on June 21, 2018 (Number 160187A 12).

The study is registered under the number NCT02884297 on clinicaltrial.gov and under the number 2015-005655-27 on EudraCT.

The research is to be conducted in accordance with the present Protocol. The Investigators undertake to respect the protocol in all respects especially with regard to obtaining consent and the notification and follow-up of serious adverse events.

Information letter and informed consent

Research participants will be informed of the objectives and constraints of the study, their rights to refuse to participate in the study, or to withdraw from the study at any time. When all essential information has been conveyed to the subject and the investigators have ensured that the patient has understood the implications of participating in the trial, the patient's written consent shall be obtained by an investigator in two original copies. A copy of the information forms and signed consents will be given to the patient.

The investigator will retain the second copy for a minimum of 15 years.

PROTOCOL AMENDMENT

The sponsor must be informed of any proposed amendment to the protocol by the coordinating investigator. The changes must be described, substantive or not.

A substantial change is a change which is susceptible, in one way or another, to modify the assurances made to participants who consent to biomedical research (modification of an inclusion criterion, extending the inclusion period, participation of new centre, etc.).

Once the research has begun, any substantial modification thereof at the initiative of the sponsor must obtain, prior to its implementation, a favourable approval of the committee and an authorisation from the competent authority. In this case, if necessary, the committee ensures that a new consent from individuals participating in research is obtained.

Any substantial change requires that an authorisation request be made by the sponsor to the ANSM and/or a notification request by the CPP in accordance with legislative regulation n ° 2004-806 of August 9, 2004.

FINAL RESEARCH REPORT

The coordinator and the mandated biostatistician will collaboratively write the final research report. This report will be submitted to each of the investigators for review. Once consensus has been reached, the final version must be endorsed with the signature of each of the investigators and sent to the sponsor as early as possible after the effective end of the research. A report prepared according to the reference plan of the competent authority must be forwarded to the competent authority and the CPP within a year after the end of the research.

DISCUSSION

This study is a follow-up to that recently published by Roberts et al.(12)

Our first objective is to confirm their data, that is, the existence of early perfusion of the intervillous space (from 8 weeks of gestation) and an increase in vascular flow starting at 13 weeks. We also seek to explore the course of vascular flow rates with gestational age to test both of the hypotheses advanced about the absence of modification of flow before 13 weeks: lack of power or external regulatory factors (remodelling of the radial arteries)?

To test the first hypothesis, we chose to increase the number of individuals in each group and to select three gestational age targets: 8, 11, and 13 weeks of gestation. A significant result between 8 and 13 weeks and between 11 and 13 weeks is expected and would thus confirm the data of Roberts et al. The intermediate group at 11 weeks was chosen arbitrarily and its size was calculated to be able to show a difference between 8 and 11 weeks.

To test the second hypothesis, and as Roberts & Frias have suggested, we will examine vascular resistance of the radial arteries at these different gestational ages and assess the level of correlation between the changes in resistance and in blood flow within the intervillous space (5,17,18).

The major difference between their study and ours is the choice of contrast product, a choice linked solely to the product available by geographic zone. They used Definity® while in Europe, we use Sonovue®. In principle this difference will have no effect on the comparison of our results, as their properties and performance are similar (19).

Moreover, we have chosen to perform 3DPD angiography at the same time to assess the applicability of this technique for the detection of blood flow before 11 weeks. The use of Doppler in the first trimester of pregnancy is a problem mainly when used with regard to the fetus. Indeed, the main hypothesis is the rise in temperature that can alter organogenesis and induce spontaneous malformations or miscarriages. In this study protocol, the acquisitions are focused on the placenta and on sections where the fetus is not visible. There is, therefore, no priori any fetal risk. Obviously, a safety assessment study will be needed before it is used in clinical practice. Should there be a high correlation between the 3DPD indices and the indicators of perfusion by CEUS, future studies will be able to choose only the 3DPD, which will make it possible to envision a longitudinal follow-up.

For placental analysis, we expect to perform a confocal microscopic analysis, immunohistochemichal analysis and extraction of RNA and specific protein. We will consider to perform also an histomorfometric analysis as Rizzo et al. (20)

Contributors: C.B. participated in study design and is carrying out or will carry out recruitment, Ultrasound acquisition, placental analysis, data analysis and manuscript writing. M.T. is carrying out or will carry out recruitment, ultrasound acquisition and data analysis. ML.E. and O.M. are major investigators for clinical assessment, Ultrasound acquisition and study design. AC and NM are project managers. GH is in charge of statistical analysis. All authors read and approved the final manuscript.

Fundings: This study won the regional award for clinical research (2016).

Competing interests: None declared

Patient consent: Obtained

Ethics approval: ANSM (the French National Agency for Medicines and Health Products Safety) and the Committee for the Protection of Persons (Comité de Protection des Personnes, CPP) approved this study.

Data sharing statement: All data generated during the project will be made freely available via CIC-IT, Nancy. Data obtained from this study will be deposited at CIC-IT Nancy where they will be maintained for a minimum of 15 years. There are no security, licensing or ethical issues related to the expected data, and all data used in the project will be generated directly as a result of the project, without any pre-existing data being used.

Trial status: This is an ongoing trial. Recruitment began in October 2016. We expect to complete recruitment by November 2019. We plan to publish final results in 2020.

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Tables and Figures

Table 1 : Inclusion and non inclusion criteria

Table 2 : Data collected

Figure 1 : Flow Chart

Figure 2 : Three dimensional Power Doppler (3DPD) Analysis

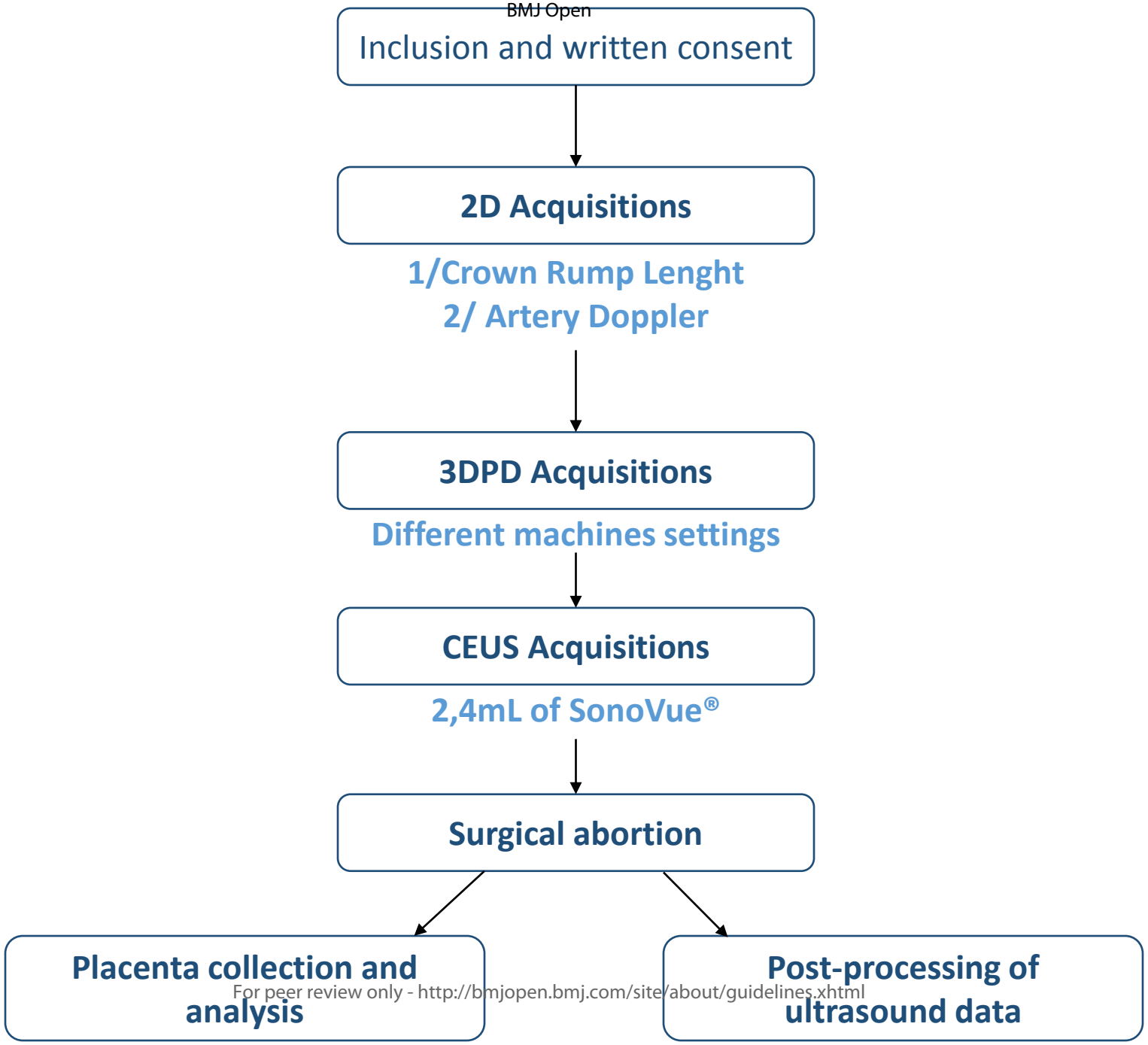
Figure 3 : Contrast-enhanced Ultrasound (CEUS) Analysis

Table 1 : Inclusion and non inclusion criteria

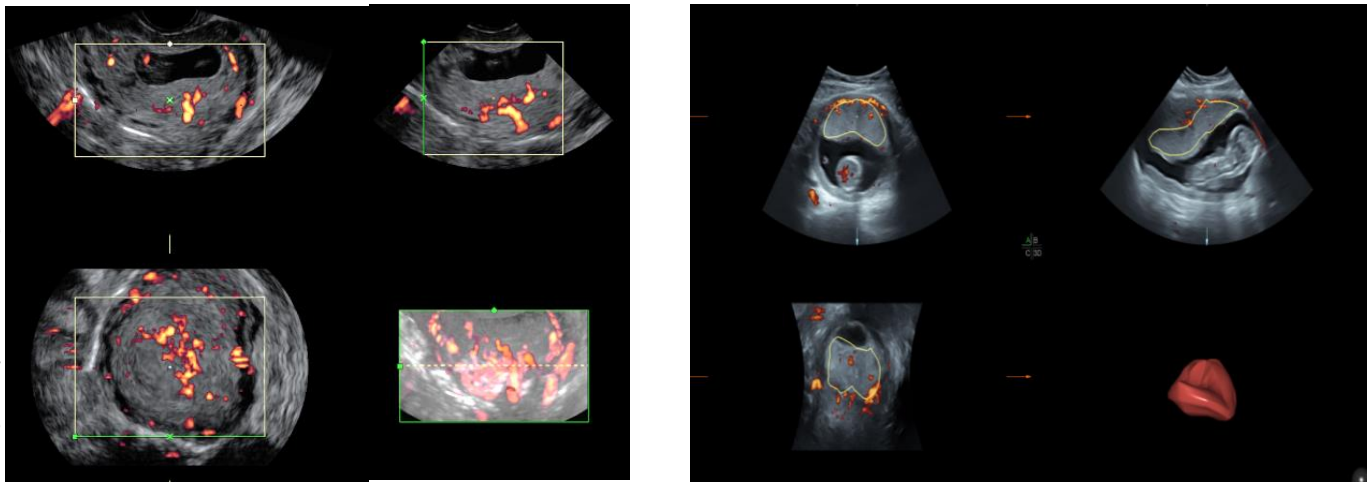
Inclusion criteria	Non inclusion criteria
- Woman age ≥ 18 years - Gestational age **8 WG Group :** 8 to 8+6 **11 WG Group :** 11 to 11+6 **13 WG Group :** 13 to 13+6 - Body Mass Index ≤ 40 kg/m² - Have given informed and written consent - Have confirmed her request of surgical elective abortion	- Contraindication to contrast injection : Hypersensitivity to sulfur hexafluoride or any of the other ingredients, history of cardiac disease, respiratory distress syndrome, severe pulmonary hypertension - Woman considered at risk of IUGR/PE : History of PE or IUGR, Autoimmune disease, Chronic High Blood Pressure, Diabetes - Woman unable to understand or follow the procedure of the study

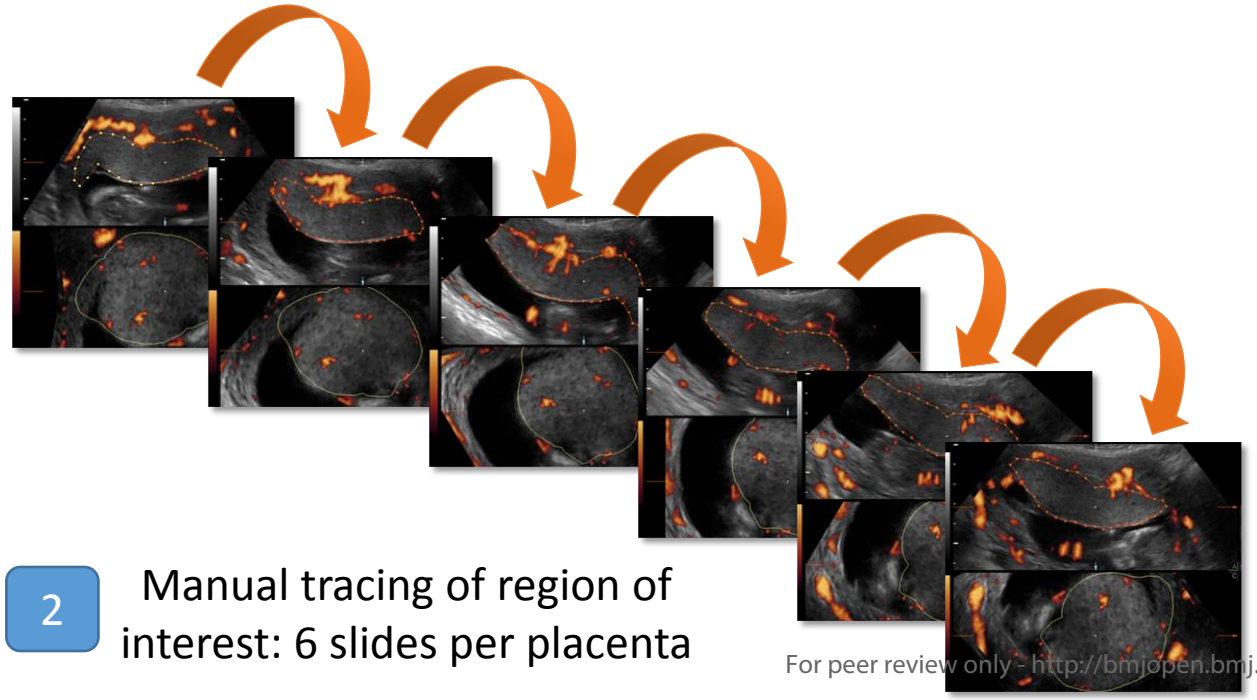
Table 2 : Data collected

Family History	Preeclampsia, intrauterine growth restriction, venous thromboembolic disease, high blood pressure, diabetes
Obstetrical History	Parity, history of post-partum hemorrhage, history of pregnancy loss or intrauterine fetal death Fibroma or uterine malformation
Clinical data	Body Mass Index Proteinuria Blood pressure (systolic and diastolic) Smoking
Current treatments	
Ultrasound and pregnancy data	Date of pregnancy ; Crown length crump (LCC), placental position, depth from the maternal abdomen, blood pressure and heart rate at the beginning of CEUS acquisition,



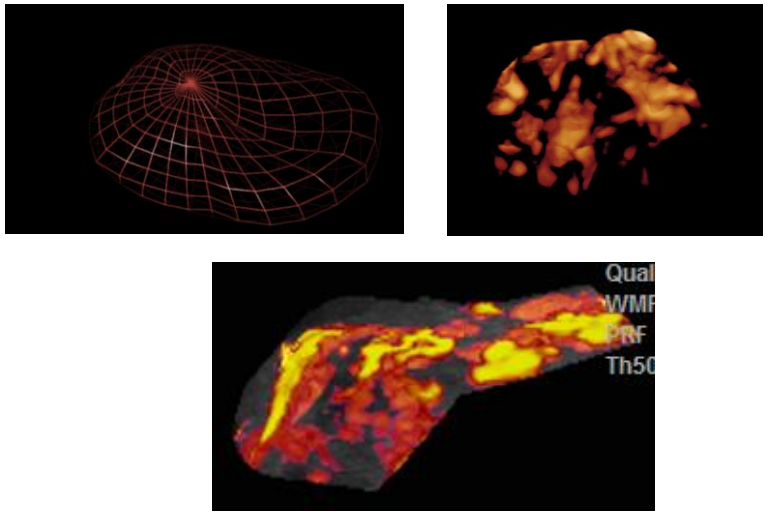
1 3DPD Acquisitions



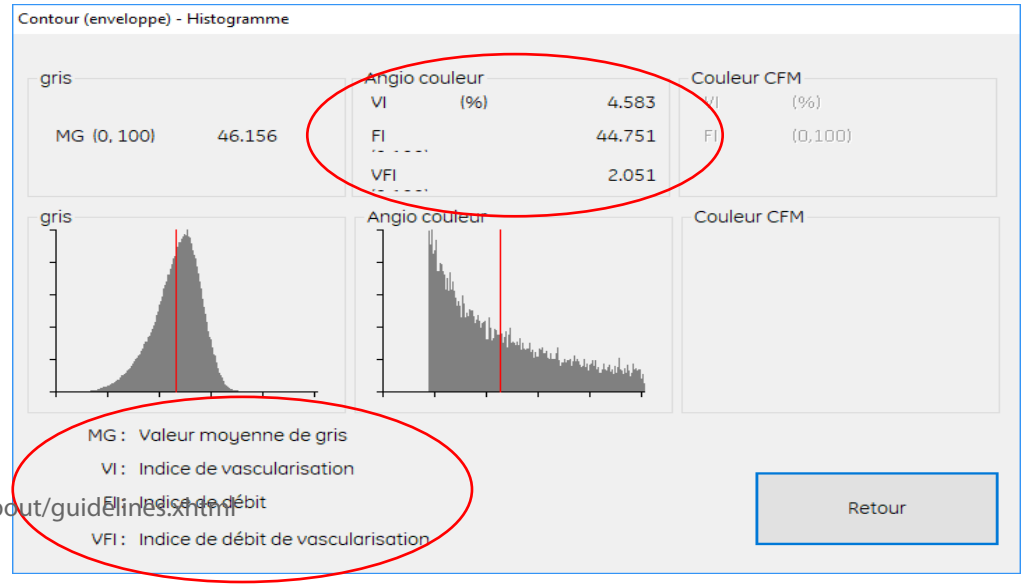


2 Manual tracing of region of interest: 6 slides per placenta

3 Placental vascularisation in 3D

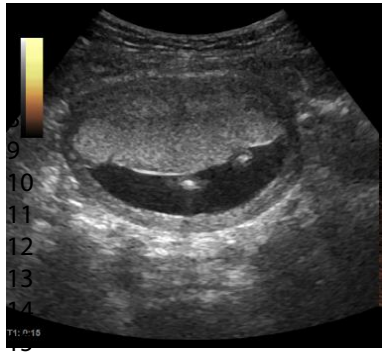


4 3DPD indices, automatically calculated

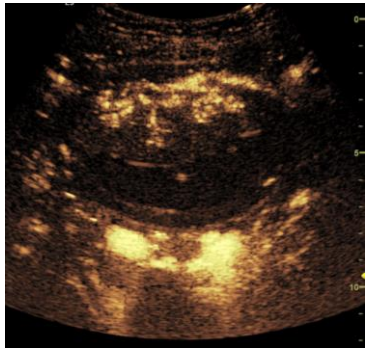


1

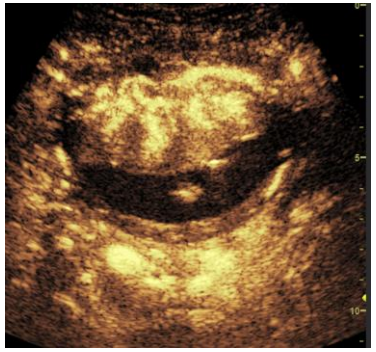
2D acquisition with contrast injection
(for exemple of 13 weeks of gestation)



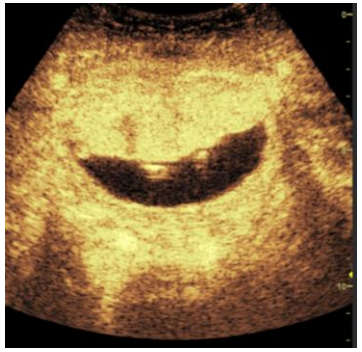
2D Without injection



With injection ; 17s



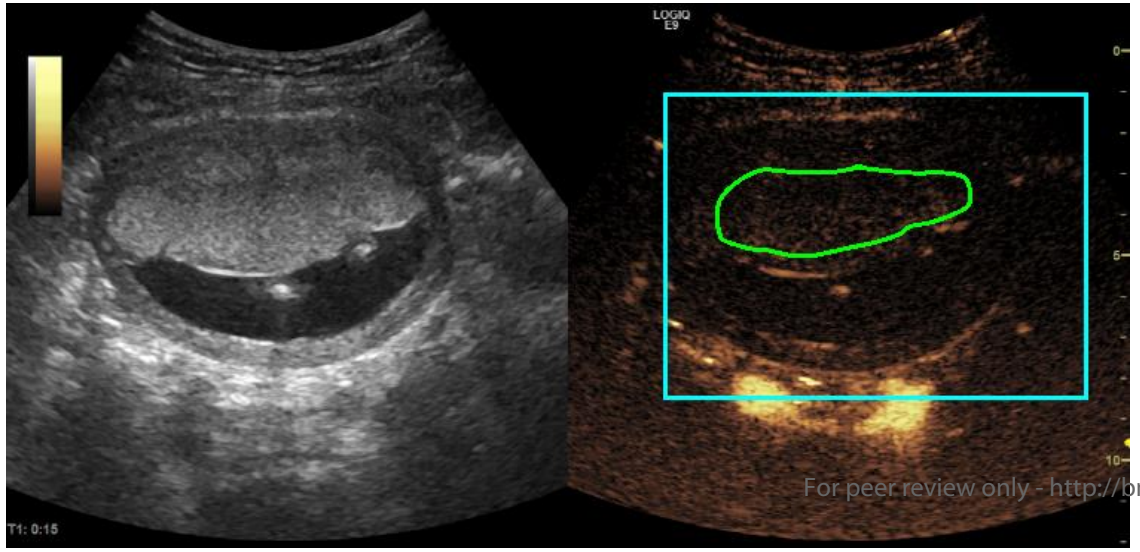
With injection ; 19s



With injection ; 27s

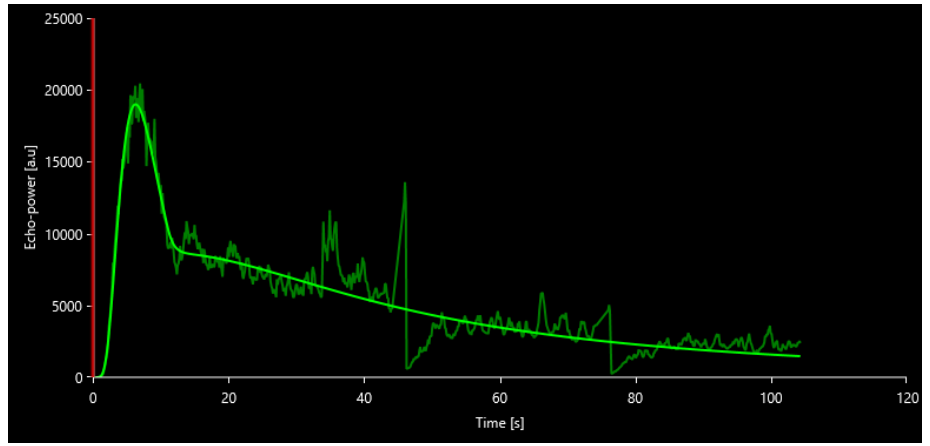
2

Manual tracing of region of interest



3

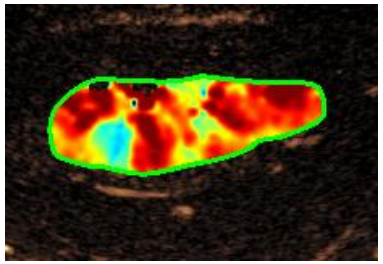
Signal intensity curves as a function of time (in secondes)



4

Extraction of semiquantitative indicators and parametric image

PARAMETERS			
PE	Peak Enhancement	ABSOLUTE	ROI1
WiAUC	Wash-in Area Under the Curve	PE[a.u]	9,86E+03
RT	Rise Time	WiAUC[a.u]	5,19E+04
mTTI	mean Transit Time (local)	RT[s]	8,065
TTP	Time To Peak	mTTI[s]	148,891
WiR	Wash-in Rate	TTP[s]	10,662
WiPI	Wash-in Perfusion Index (WiAUC / I	WiR[a.u]	1,88E+03
WoAUC	Wash-out AUC	WiPI[a.u]	6,43E+03
WiWoAUC	Wash-in and Wash-out AUC	WoAUC[a.u	1,30E+05
FT	Fall Time	WiWoAUC[	1,82E+05
WoR	Wash-out Rate	FT[s]	19,599
QOF	Quality Of Fit	WoR[a.u]	5,51E+02
Area	ROI Area	QOF[%]	89,227
		Area[cm2]	4,662



Parametric image of Peak enhancement

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
page 1 Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
p 9 Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
p11 Protocol version	3	Date and version identifier
p 11 Funding	4	Sources and types of financial, material, and other support
p 1 Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
p 11	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
p3-4 Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
p4 Objectives	7	Specific objectives or hypotheses
p4 Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis			
p6-7-15	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
p6-7	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
p7	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring			
p8	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Assessment of uteroplacental vascularisation in early first-trimester pregnancy with contrast-enhanced ultrasound and 3D power Doppler angiography: protocol for a prospective, cross-sectional, multicentre, non-randomised open study (HOPE).

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-030353.R2
Article Type:	Protocol
Date Submitted by the Author:	11-Jul-2019
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Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Radiology and imaging
Keywords:	Ultrasonography < OBSTETRICS, Prenatal diagnosis < OBSTETRICS, Ultrasound < RADIOLOGY & IMAGING, Physiology < BASIC SCIENCES, Pathology < BASIC SCIENCES

Assessment of uteroplacental vascularisation in early first-trimester pregnancy with contrast-enhanced ultrasound and 3D power Doppler angiography: protocol for a prospective, cross-sectional, multicentre non-randomised open study (HOPE).

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Abstract

Introduction: Knowledge about the mechanisms leading to the establishment of uteroplacental vascularisation is inadequate, and some of what has been thought to be known for decades has recently been challenged by a showing that the intervillous space, the major area of maternal-fetal exchange, appears to be perfused by maternal blood at as early as 6 weeks of gestation. The vascular flow then seems relatively constant until 13 weeks, when it appears to increase suddenly.

Objectives: The principal objective is to quantify the perfusion of the intervillous space by contrast-enhanced ultrasonography (CEUS) during the first-trimester at three different gestational ages (8, 11, and 13 weeks). The secondary objectives are to: (i) describe the indicators of vascularisation of the placenta (intervillous space) and the myometrium at the three gestational ages, measured by CEUS and 3D power Doppler (3DPD) angiography; (ii) compare the diagnostic performance of CEUS and 3DPD for the demonstration and quantification of uteroplacental vascularisation; and (iii) establish a biological collection of placentas to increase knowledge about placental development and functions during pregnancy.

Methods and Analysis: This is a prospective, cross-sectional, multicentre, non-randomised, open study. We will include 42 women with ongoing pregnancy and divided into three group of gestational ages (that is, 14 women by per group): 8, 11, and 13 weeks of gestation. 3DPD and then CEUS will be performed and the data about the perfusion kinetics and the 3DPD indices will be calculated and then compared with each other and for each gestational age.

Ethics and Dissemination: The appropriate French Ethics Committee Est III approved this study and the related consent forms on April 5, 2016, and the competent authority (ANSM) authorised the study on June 21, 2016. The results of this study will be published in a peer-reviewed journal and will be presented at relevant conferences.

Strengths and limitations of this study

- The recent data will be confirmed in our study with a larger number of women.
- To our knowledge, this will be the first study comparing 3DPD with CEUS. Should the results demonstrate the value of 3DPD, studies with a longitudinal follow-up, that is, of ongoing pregnancies, could be conducted and would enable imaging data to be correlated with final pregnancy outcomes.
- The groups are selected by gestational age.
- The pathophysiologic interpretation of the data will be limited by the absence of information about pregnancy outcome.

INTRODUCTION

Preeclampsia and intrauterine growth restriction (IUGR) are two principal complications of pregnancy that account for more than 30% of maternal and fetal morbidity and mortality. These diseases, which affect from 4 to 7% of pregnancies, are related to chronic uteroplacental hypoperfusion, and knowledge of its pathophysiologic mechanisms remains inadequate. Currently, the major hypothesis is that defective trophoblastic invasion during the first trimester leads to chronic uteroplacental hypoperfusion (1).

The mechanisms that establish uteroplacental vascularisation during the first trimester were described several decades ago, based on pathology studies, in the absence of functional imaging tools applicable *in vivo* in pregnant women(2,3). The presence of trophoblastic (also called endovascular) plugs in the spiral arteries was thought to prevent perfusion of the intervillous space until approximately 10 weeks of gestation, to enable the trophoblast to develop in a favourable hypoxic situation. More recently, ultrasound exploration seemingly confirming the absence of a Doppler signal before 12 weeks strengthened this hypothesis(4,5). Other work, notably histologic exploration of early-pregnancy hysterectomy specimens suggesting the absence of vascular "connection" before 8 weeks of gestation between the maternal network and the intervillous space and physiological explorations showing the maintenance of a hypoxic environment within the placenta up to 10 weeks of gestation also pointed in this direction at the beginning of this century (2,6).

On the other hand, the chronology and conditions leading to the disappearance of these plugs remained unknown. Based on this knowledge, it was suggested the origin of the chronic placental hypoperfusion phenomena observed in preeclampsia and intrauterine growth restriction might be the premature disappearance of these plugs and therefore the loss of the hypoxic environment (7–11).

This entire set of pathophysiologic hypotheses was called into question in 2017. Specifically, Roberts et al. (*Hum Reprod*, 2017) applied a modern technique of functional imaging — contrast-enhanced ultrasonography (CEUS) — to the placenta to show that the intervillous space was perfused by 6 weeks of gestation(12).They suggested in particular that the plugs disappear between 6 and 8 weeks.

This exploratory work thus presents major interest in terms of the physiological understanding of uteroplacental perfusion in the first trimester. Nonetheless, the study design and the number of pregnancies studied did not make it possible to go farther in understanding the quantitative course of this perfusion of the intervillous space. Accordingly, the authors observed quantitative modification of vascular flow within the intervillous space only starting at 13 weeks; they accordingly hypothesised that late remodelling of the radial arteries led to the reduction of vascular resistance. It is nonetheless also possible that the study lacked the power to show quantitative differences in vascular flow between 6 and 13 weeks.

These new data must imperatively be confirmed by other studies with more subjects, designed in principle with the objective of quantifying flow as a function of gestational age. The

demonstration of blood within the intervillous space at 6 weeks of gestation was possible through the use of an ultrasound contrast product, known to be strictly intravascular.

3D power Doppler (3DPD) angiography is another innovative technique for functional placental imaging. First described in 2004, it has the major interest of being usable in pregnant women because it has no teratogenic risk(13). It appears to present a major potential interest for the study of pathophysiologic phenomena associated with uteroplacental vascularisation(14,15). This technique, however, has only been assessed at a gestational age of 11 weeks (16). Its principal limitations are the absence of absolute specificity of the Doppler signal for blood flow and the impossibility of differentiating maternal and fetal flow. Accordingly, it would be interesting to assess whether 3DPD angiography might make it possible to quantify early perfusion of the intervillous space, by comparing it with CEUS, which is specific for maternal blood flow. If this is the case, contrast product would no longer be necessary for exploring uteroplacental vascularisation during pregnancy.

OBJECTIVE

Primary objective

The principal objective is to quantify the perfusion of the intervillous space by CEUS during the first trimester at three different gestational ages: 8, 11, and 13 weeks.

Secondary objectives

- Describe the indicators of vascularisation of the placenta (intervillous space) and the myometrium at the three gestational ages, measured by CEUS and 3DPD.

- Compare the diagnostic performance of CEUS and 3DPD for the demonstration and quantification of uteroplacental vascularisation.

- Establish a biological collection of placentas to increase knowledge about placental development and functions during pregnancy.

METHODS

Trial design

Prospective, physiological, cross-sectional, multicentre, and non-randomised open study. This is a multicentre study including a level III university hospital centre (CHRU Nancy) and a level II regional hospital centre (CHR Metz).

Study population

Table 1 presents the inclusion and non inclusion criteria and Figure 1 presents the flow chart of the study.

The participants in this study are women with ongoing pregnancy at the first-trimester. The analysis of uteroplacental vascularisation by CEUS can only be performed in contexts of elective abortion, as the contrast product is not authorised for use in ongoing pregnancies.

Before inclusion, the women will be informed of the aim, the procedures, and the predictable risks of the study (no identified clinical risk) by the principal investigator.

US acquisition

The ultrasound acquisition will begin in 2D mode by measurement of the crown-rump length to verify gestational age and study group. The position of the placenta will be noted and the Doppler spectrum of the uterine arteries, spiral arteries, and radial arteries will be recorded, with measurements of the pulsatility and resistance indices.

First, the 3D Doppler acquisitions will be performed at 4 different predetermined settings (impact of the settings currently underway: NCT03342014). The instrument used will be a Voluson S8 (General Electric Healthcare) with a volumetric convex abdominal transducer (4–8 MHz).

Next, the CEUS acquisitions will be performed with a Logiq E9 (General Electric Healthcare) and an abdominal transducer (1-5 MHz). The ultrasound contrast product used will be Sonovue® (Bracco Imaging, Italy), administered by bolus injection. A volume of 2.4 mL of contrast product will be injected per patient, and repeated once if necessary.

Settings and equipments are standardized between the two centers.

Image analysis

3DPD angiography: The images will be analysed with VOCAL® software, which makes it possible to define a volume of interest and to quantify the Doppler signals to calculate the vascularisation indices (VI, FI, and VFI) automatically (Figure 2). Each volume will be recorded and analysed independently. Two regions of interest will be traced: one focused on the placenta and the other on the myometrium (placental bed).

CEUS: The images will be analysed with specific software that makes it possible to trace the regions of interest and to view the perfusion curves and to extract semiquantitative perfusion indicators from them (Figure 3).

Placenta collection and analyses

The placentas will be collected and transferred fresh for preparation. The placental villi will be isolated and rinsed in saline solution. A portion of the villi (approximately 1 to 2 cm^3 of tissue) will be frozen at -80°C for the subsequent extraction of RNA and specific proteins. For an immunohistochemical analysis, a second portion will be fixed in buffered formalin for inclusion in paraffin and serial sections of 5-µm will be made on superfrost slides. The markers of oxidative stress will be specifically studied. The third portion will be fixed in formalin, diluted à 4%, and kept at 4°C in order to perform a confocal microscopic analysis.

All the villi will be collected and we consider that it is representative sampling of the placenta. However, it will be not possible to perform a spatial localization of the villi because placenta if surgically collected and as multiples parts.

Outcomes

The principal endpoint is the measurement (in arbitrary units) of signal intensity in the intervillous space during the first trimester (at 8, 11, and 13 weeks), obtained by CEUS.

The secondary outcome measures are:

1. Measurement of indicators of vascularisation in the intervillous space and the myometrium: signal intensity and perfusion kinetics.
2. Comparison of the quantitative data about uteroplacental vascularisation obtained with each technique.
3. Procurement of placental villi that can be analysed to study human placental development and functions.

Participant timeline

The enrolment of women began in October 2016. In view of the recruitment capacity of our institutions, the recruitment should be completed by November 2019.

Patient and public involvement

Patients and public were not involved.

Premature ending of patient participation

Participants will be excluded from the study in the following situations:

- Lack of CEUS acquisition
- Withdrawal of consent before the end of the study.

Patients will be immediately excluded from the study and replaced with other new participants. Any decision to withdraw consent will not affect the patient's routine medical care. In the case of an adverse event related to the study, the patient will be informed and excluded. She will also receive what additional medical care is necessary or appropriate.

Follow-up

No specific follow-up has been planned for participants except for standard routine healthcare. Any adverse events will be noted and reported.

Sample size consideration

In view of recent data from the literature (Roberts, *Hum Reprod*. 2017), to show a difference in signal intensity between the three groups (8, 11, and 13 weeks) with α (corrected for the

multiple comparison) and β risks set respectively at 0.017 and 10%, we should have 14 women in each group at analysis, that is, 42 women in all.

Data collection and management:

An electronic case report file (e-CRF) will be created for each woman. The women's anonymity will be ensured by mentioning to the maximum extent possible their research code number, followed by the first letters of the last name and first name of the participant on all necessary documents or by deleting their names by appropriate means (white-out) from the copies of source documents intended to document the study.

The ultrasound data will be anonymised and transferred via a secure server for storage and archiving directly in the ARCHIMED database, reported to the CNIL (CNIL report number: 1410005) because they cannot be transcribed in the e-CRF. The clinical data concerning the woman collected are in Table 2.

Statistical analysis

The quantitative indicators will be described by their means ± standard deviations, medians, and maximum and minimum values, the qualitative indicators by the number of individuals and percentages. The mean values will be compared between the groups by Student's t test or the Mann-Whitney test, matched or not, depending on the type of data. The comparison of imaging techniques will be completed by Bland-Altman plots.

The analyses will be performed with R software.

No interim analysis is planned.

No statistical criterion for stopping the study is planned.

QUALITY CONTROL

Right of access to data and source documents

The medical data of each patient will only be transmitted to the sponsor (Metz-Thionville Regional Hospital Centre, CHR Metz-Thionville) or any person duly authorised by the sponsor and, where applicable, to the authorised health authorities, under conditions guaranteeing their confidentiality.

The sponsor and the regulatory authorities may request direct access to the medical file for verification of the procedures and/or data of the clinical trial, without violating confidentiality and within the limits permitted by laws and regulations.

For research purposes, the processing of personal data relating to persons undergoing research will be carried out.

These data are collected and processed solely on the basis of the legal grounds provided for by statute and regulations in the context of the performance of the public interest missions of the Metz-Thionville CHR, in particular those relating to ensuring and contributing to research

and innovation (Article 6.1.e of the GDPR, General Data Protection Regulation). The processing of personal data of persons participating in research is permitted by the exception provided for in Article 9.2(i) and (j).

This data processing is part of the MR001 reference methodology that the Metz-Thionville CHR has undertaken to respect.

In accordance with the GDPR, persons participating in research have a right of access to their data (Article 15), a right of rectification (Article 16), a right to erase their data (right to forget) under the conditions provided for in Article 17, a right to limit the processing provided for in Article 18, and a right to object to the processing of their personal data (Article 21). These rights are exercised with the Investigators, who will inform the research sponsor as soon as possible.

The persons participating in the research also have a right to complain to the supervisory authority in France, namely, the Commission Nationale de l'Informatique et des Libertés (CNIL).

Study monitoring

The monitoring will be performed by the appropriate department of the Support Platform for Clinical Research (PARC) of CHR Metz-Thionville, throughout the study.

A Clinical Research Associate (CRA) will travel regularly to each centre to perform the quality control of the e-CRF data.

The CRA will verify that the research is conducted according to the protocol provided in accordance with regulations and will ensure that every e-CRF contains all of the information requested.

Each patient's e-CRF must be consistent with the source documents.

The investigators will allocate adequate time for such monitoring activities. The investigators will also make sure that the monitor or other compliance or quality assurance reviewer is given access to all of the above noted study-related documents and study-related facilities (e.g. Ultrasound, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visits.

Data management and quality control

Data management will be carried out by the clinical research team CIC-IT of Nancy (INSERM CIC 1433). The data images (ultrasound) will be automatically transferred to Nancy CIC-IT and stored after verification in the ArchiMed database declared to the French authorities (CNIL declaration number: 1410005).

Patient data protection

Each patient must be identified on the e-CRF, ultrasound data, and placenta collection with her initials and identification number indicating her order of inclusion into the study. The

investigators must keep the list of all the patients, including identification numbers, full names and last known addresses.

Patients must be informed in writing about the possibility of audits by authorised representatives of the sponsor and/or regulatory authorities, in which case the relevant parts of study-related hospital records may be required.

Patients must also be informed that the results obtained will be computerised and analysed, that local laws will be applied, that their confidentiality will be preserved, and that they are entitled to obtain any information concerning the data stored and analysed by the computerised system.

POTENTIAL RISKS RELATED TO THE STUDY

This study may expose the women participating in it to rare, transient, mild side effects. It will comply at all times with the Good Clinical Practices defined by the Ministry of Health.

The only constraint associated with the study is the addition of 3D Doppler acquisitions after the standard 2D ultrasound; these acquisitions will prolong the procedure by 10 minutes. The medical devices (Voluson® and Logiq E9®, GE Healthcare) are CE-marked and used routinely in clinical practice.

The contrast product (SonoVue®) is authorised for use in exploring lesions of the liver, breasts, and great vessels. It will be used according to the guidelines described in the summary of product characteristics in its French marketing authorisation. In the current state of knowledge about the safety of contrast products (which have not yet been authorised for use during pregnancy), adequate evidence exists to affirm their absence of permanent or serious adverse effects among women.

ETHICAL PERMISSION

The sponsor and all investigators undertake to conduct this study in accordance with the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects, Tokyo 2004) and its updates, the provisions of European Directive 2001/20-CE as transposed into French law by L. 2004-806 dated August 9, 2004, on public health policy and 2004-800 dated August 6, 2004, on bioethics and their implementation decrees, and to comply with the guidelines of Good Clinical Practices (I.C.H. version 4 of May 1, 1996 and Decision of November 24, 2006).

They undertake to adhere to all legislative and regulatory provisions that may concern the research. In accordance with Article L. 1123-6 of the Public Health Code, the sponsor has submitted the research protocol the sponsor to the appropriate Committee for the Protection of Persons (CPP, Patient Protection Committee) Est III, which approved this study and the related consent forms on April 5, 2018 (Number 16.03.02). The competent authority (ANSM, Agence Nationale de Sécurité du Médicament et des Produits de Santé) authorised the study on June 21, 2018 (Number 160187A 12).

The study is registered under the number NCT02884297 on clinicaltrial.gov and under the number 2015-005655-27 on EudraCT.

The research is to be conducted in accordance with the present Protocol. The Investigators undertake to respect the protocol in all respects especially with regard to obtaining consent and the notification and follow-up of serious adverse events.

Information letter and informed consent

Research participants will be informed of the objectives and constraints of the study, their rights to refuse to participate in the study, or to withdraw from the study at any time. When all essential information has been conveyed to the subject and the investigators have ensured that the patient has understood the implications of participating in the trial, the patient's written consent shall be obtained by an investigator in two original copies. A copy of the information forms and signed consents will be given to the patient (Annexe 1).

The investigator will retain the second copy for a minimum of 15 years.

PROTOCOL AMENDMENT

The sponsor must be informed of any proposed amendment to the protocol by the coordinating investigator. The changes must be described, substantive or not.

A substantial change is a change which is susceptible, in one way or another, to modify the assurances made to participants who consent to biomedical research (modification of an inclusion criterion, extending the inclusion period, participation of new centre, etc.).

Once the research has begun, any substantial modification thereof at the initiative of the sponsor must obtain, prior to its implementation, a favourable approval of the committee and an authorisation from the competent authority. In this case, if necessary, the committee ensures that a new consent from individuals participating in research is obtained.

Any substantial change requires that an authorisation request be made by the sponsor to the ANSM and/or a notification request by the CPP in accordance with legislative regulation n ° 2004-806 of August 9, 2004.

FINAL RESEARCH REPORT

The coordinator and the mandated biostatistician will collaboratively write the final research report. This report will be submitted to each of the investigators for review. Once consensus has been reached, the final version must be endorsed with the signature of each of the investigators and sent to the sponsor as early as possible after the effective end of the research. A report prepared according to the reference plan of the competent authority must be forwarded to the competent authority and the CPP within a year after the end of the research.

DISCUSSION

This study is a follow-up to that recently published by Roberts et al.(12)

Our first objective is to confirm their data, that is, the existence of early perfusion of the intervillous space (from 8 weeks of gestation) and an increase in vascular flow starting at 13 weeks. We also seek to explore the course of vascular flow rates with gestational age to test both of the hypotheses advanced about the absence of modification of flow before 13 weeks: lack of power or external regulatory factors (remodelling of the radial arteries)?

To test the first hypothesis, we chose to increase the number of individuals in each group and to select three gestational age targets: 8, 11, and 13 weeks of gestation. A significant result between 8 and 13 weeks and between 11 and 13 weeks is expected and would thus confirm the data of Roberts et al. The intermediate group at 11 weeks was chosen arbitrarily and its size was calculated to be able to show a difference between 8 and 11 weeks.

To test the second hypothesis, and as Roberts & Frias have suggested, we will examine vascular resistance of the radial arteries at these different gestational ages and assess the level of correlation between the changes in resistance and in blood flow within the intervillous space (5,17,18).

The major difference between their study and ours is the choice of contrast product, a choice linked solely to the product available by geographic zone. They used Definity® while in Europe, we use Sonovue®. In principle this difference will have no effect on the comparison of our results, as their properties and performance are similar (19).

Moreover, we have chosen to perform 3DPD angiography at the same time to assess the applicability of this technique for the detection of blood flow before 11 weeks. The use of Doppler in the first trimester of pregnancy is a problem mainly when used with regard to the fetus. Indeed, the main hypothesis is the rise in temperature that can alter organogenesis and induce spontaneous malformations or miscarriages. In this study protocol, the acquisitions are focused on the placenta and on sections where the fetus is not visible. There is, therefore, no priori any fetal risk. Obviously, a safety assessment study will be needed before it is used in clinical practice. Should there be a high correlation between the 3DPD indices and the indicators of perfusion by CEUS, future studies will be able to choose only the 3DPD, which will make it possible to envision a longitudinal follow-up.

For placental analysis, we expect to perform a confocal microscopic analysis, immunohistochemichal analysis and extraction of RNA and specific protein. We will consider to perform also an histomorfometric analysis as Rizzo et al. (20)

Contributors: C.B., ML.E. and O.M. designed the study and are the principal investigators; A.C. and N.M. are project managers; C.B. wrote the manuscript; C.B. and M.T. will carry out recruitment, ultrasound acquisition, placental analysis and will collect the data; G.H. is in charge of statistical analysis and all authors reviewed and contributed to the manuscript.

All authors have read, approved the paper and meet the criteria for authorship as established by the International Committee of Medical Journals Editors.

Fundings: This study won the regional award for clinical research (2016).

Competing interests: None declared

Patient consent: Obtained

Ethics approval: ANSM (the French National Agency for Medicines and Health Products Safety) and the Committee for the Protection of Persons (Comité de Protection des Personnes, CPP) approved this study.

Data sharing statement: All data generated during the project will be made freely available via CIC-IT, Nancy. Data obtained from this study will be deposited at CIC-IT Nancy where they will be maintained for a minimum of 15 years. There are no security, licensing or ethical issues related to the expected data, and all data used in the project will be generated directly as a result of the project, without any pre-existing data being used.

Trial status: This is an ongoing trial. Recruitment began in October 2016. We expect to complete recruitment by November 2019. We plan to publish final results in 2020.

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Tables and Figures

Table 1 : Inclusion and non inclusion criteria

Table 2 : Data collected

Figure 1 : Flow Chart

Figure 2 : Three dimensional Power Doppler (3DPD) Analysis

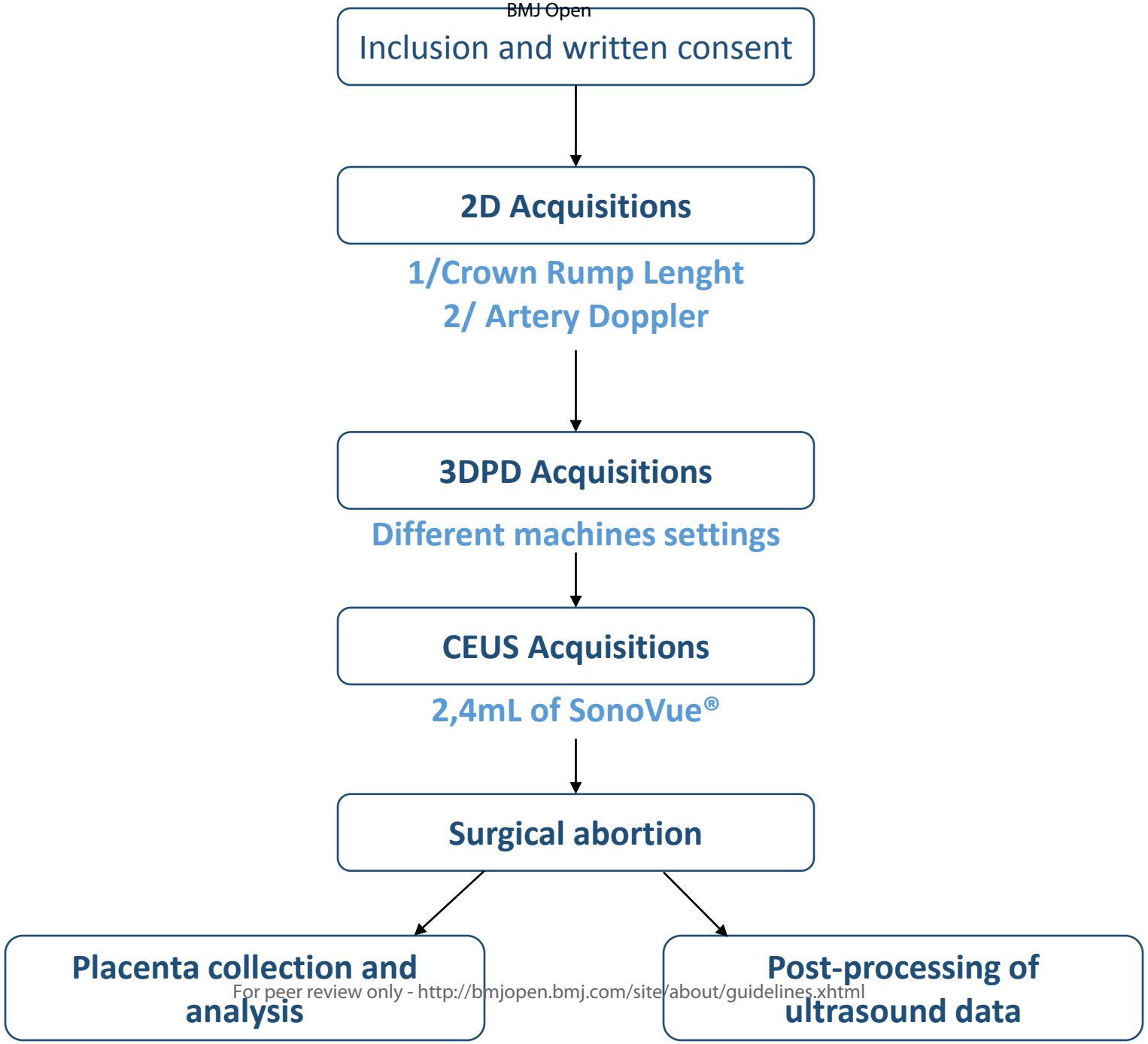
Figure 3 : Contrast-enhanced Ultrasound (CEUS) Analysis

Table 1 : Inclusion and non inclusion criteria

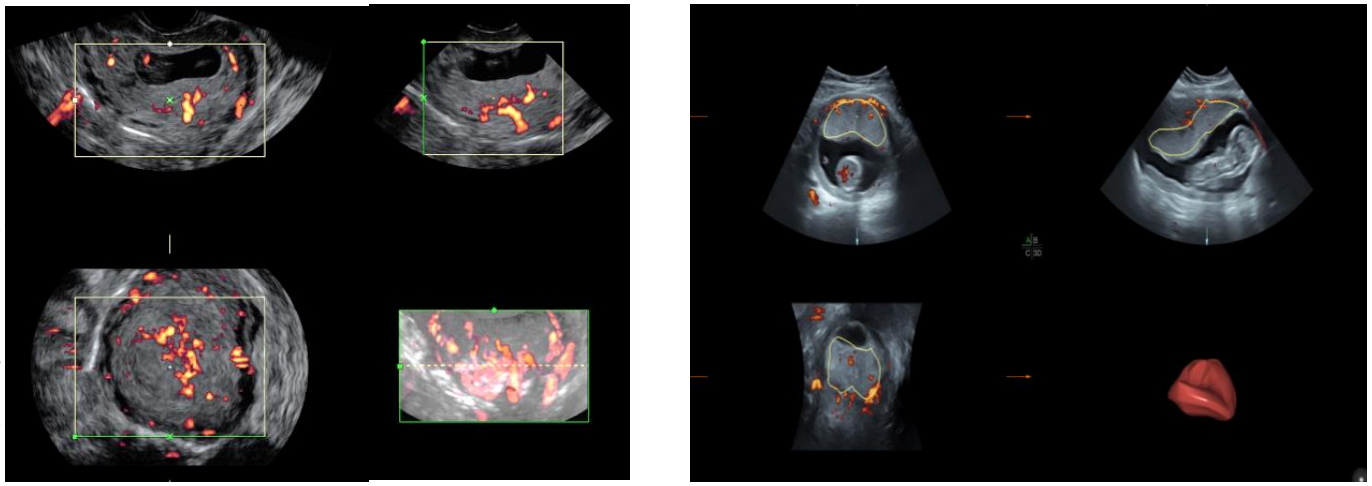
Inclusion criteria	Non inclusion criteria
- Woman age ≥ 18 years - Gestational age **8 WG Group :** 8 to 8+6 **11 WG Group :** 11 to 11+6 **13 WG Group :** 13 to 13+6 - Body Mass Index ≤ 40 kg/m² - Have given informed and written consent - Have confirmed her request of surgical elective abortion	- Contraindication to contrast injection : Hypersensitivity to sulfur hexafluoride or any of the other ingredients, history of cardiac disease, respiratory distress syndrome, severe pulmonary hypertension - Woman considered at risk of IUGR/PE : History of PE or IUGR, Autoimmune disease, Chronic High Blood Pressure, Diabetes - Woman unable to understand or follow the procedure of the study

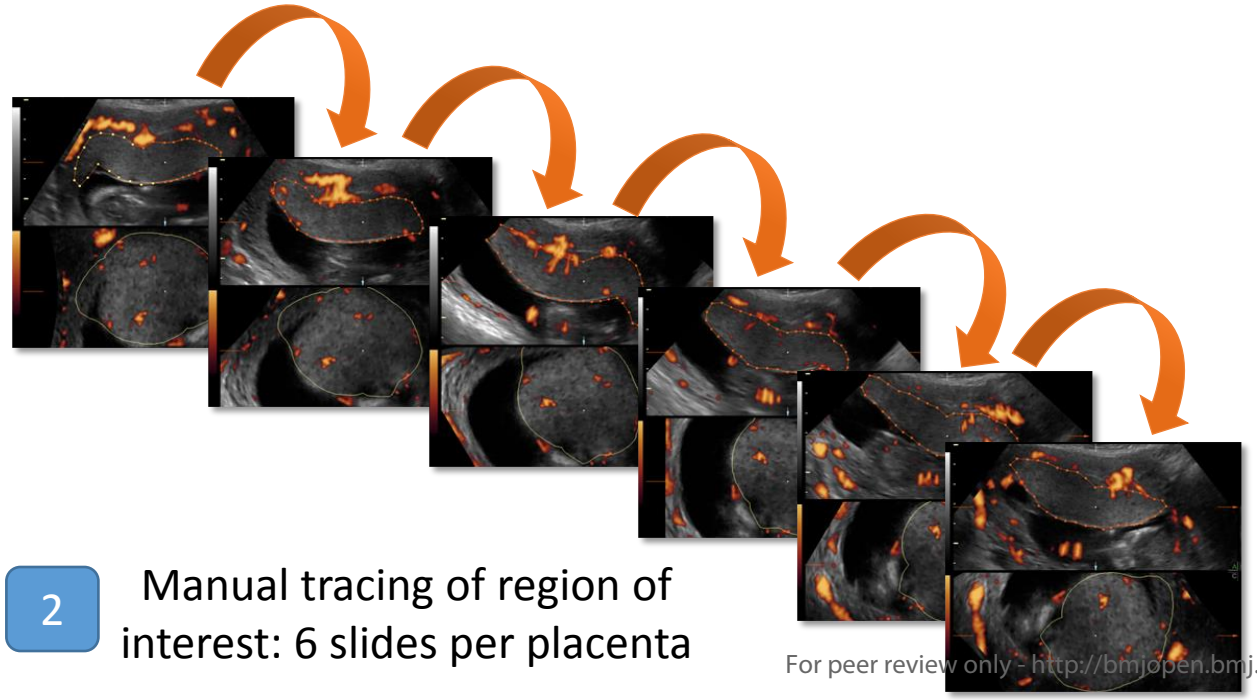
Table 2 : Data collected

Family History	Preeclampsia, intrauterine growth restriction, venous thromboembolic disease, high blood pressure, diabetes
Obstetrical History	Parity, history of post-partum hemorrhage, history of pregnancy loss or intrauterine fetal death Fibroma or uterine malformation
Clinical data	Body Mass Index Proteinuria Blood pressure (systolic and diastolic) Smoking
Current treatments	
Ultrasound and pregnancy data	Date of pregnancy ; Crown length crump (LCC), placental position, depth from the maternal abdomen, blood pressure and heart rate at the beginning of CEUS acquisition,



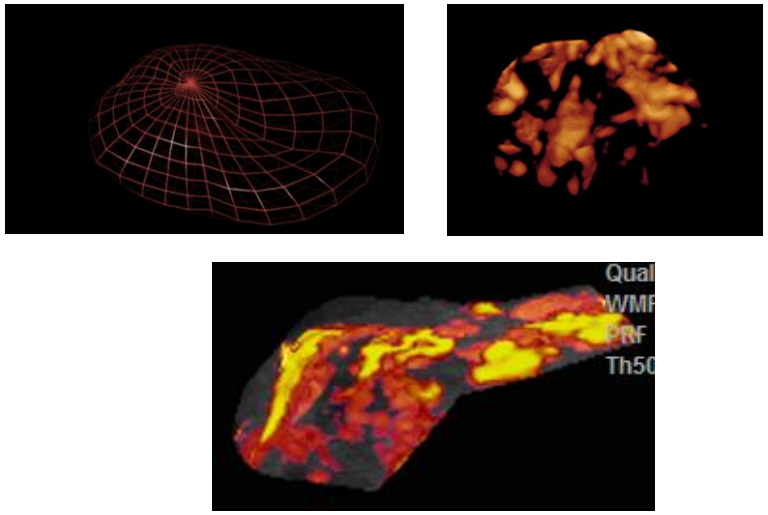
1 3DPD Acquisitions



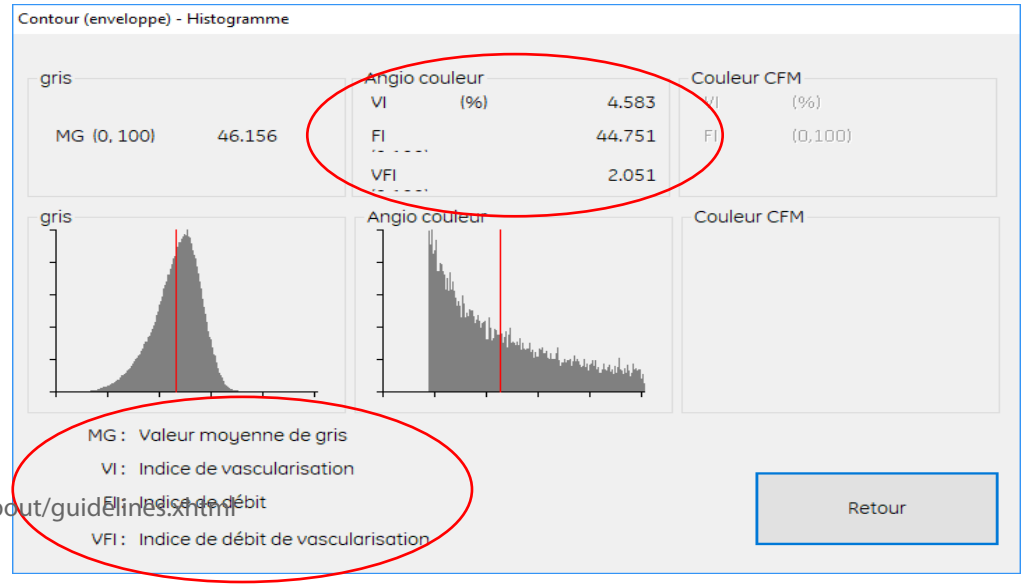


2 Manual tracing of region of interest: 6 slides per placenta

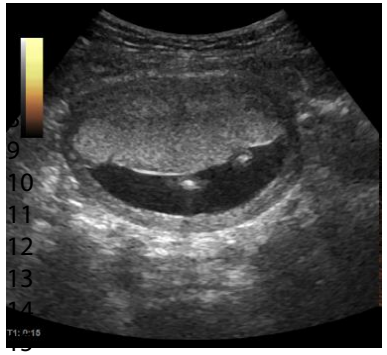
3 Placental vascularisation in 3D



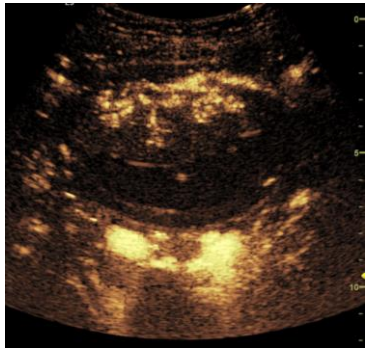
4 3DPD indices, automatically calculated



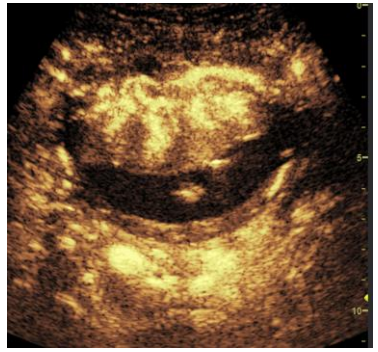
1 2D acquisition with contrast injection
(for exemple of 13 weeks of gestation)



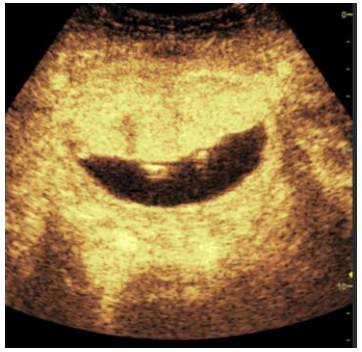
2D Without injection



With injection ; 17s

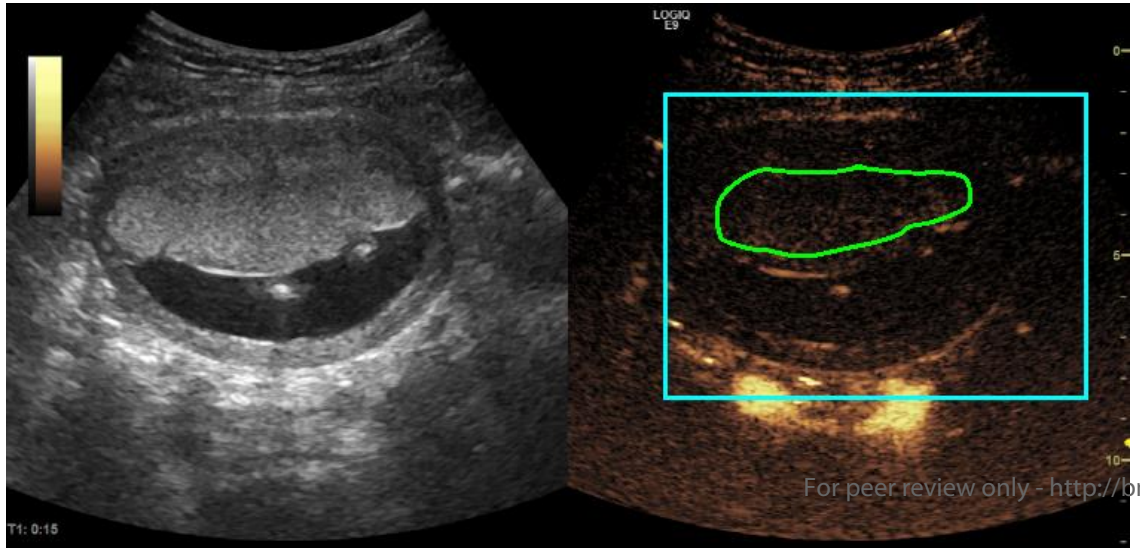


With injection ; 19s

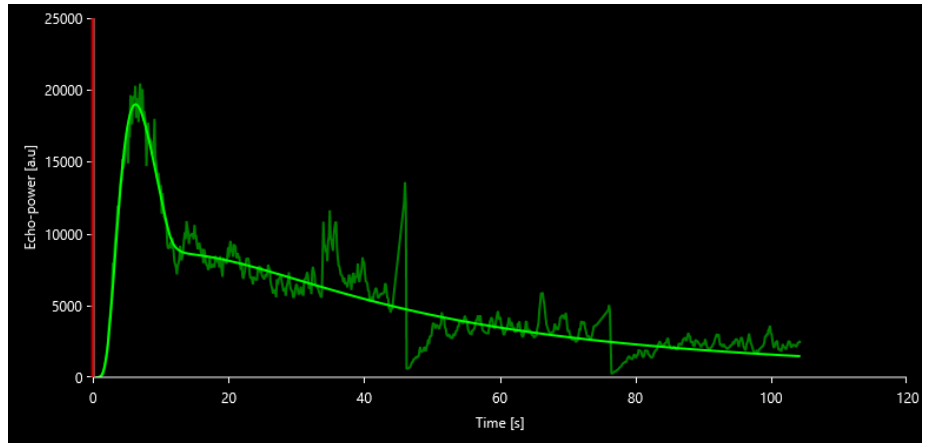


With injection ; 27s

2 Manual tracing of region of interest

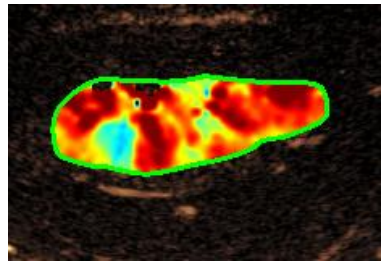


3 Signal intensity curves as a function of time (in secondes)



4 Extraction of semiquantitative indicators and parametric image

PARAMETERS			
PE	Peak Enhancement	ABSOLUTE	ROI1
WiAUC	Wash-in Area Under the Curve	PE[a.u]	9,86E+03
RT	Rise Time	WiAUC[a.u]	5,19E+04
mTTI	mean Transit Time (local)	RT[s]	8,065
TTP	Time To Peak	mTTI[s]	148,891
WiR	Wash-in Rate	TTP[s]	10,662
WiPI	Wash-in Perfusion Index (WiAUC / I	WiR[a.u]	1,88E+03
WoAUC	Wash-out AUC	WiPI[a.u]	6,43E+03
WiWoAUC	Wash-in and Wash-out AUC	WoAUC[a.u	1,30E+05
FT	Fall Time	WiWoAUC[	1,82E+05
WoR	Wash-out Rate	FT[s]	19,599
QOF	Quality Of Fit	WoR[a.u]	5,51E+02
Area	ROI Area	QOF[%]	89,227
		Area[cm2]	4,662



Parametric image of Peak enhancement

PATIENT CONSENT FORM

Details about this study are provided in the specific newsletter provided to you.
Read this manual carefully and ask all the questions that you think will be useful.
If you agree to participate in this study, please complete the form below.

Title of the study : Assessment of uteroplacental vascularisation in early first-trimester pregnancy with contrast-enhanced ultrasound and 3D power Doppler angiography: protocol for a multicentre prospective study (HOPE).

SPONSOR : Centre Hospitalier Régional (CHR) de Metz-Thionville
CHR-METZ-THIONVILLE
Hôpital de Mercy 1, Allée du Château - CS 45001
57085 Metz Cedex 03.

I, the undersigned, Mrs. .... (full name in capital letters), declares to have understood the purpose and the modalities of this study, which were fully explained to me by the Doctor .... (full name in capital letters), investigator doctor. I received the specific information form that I had the opportunity to study carefully.
Answers have been made to all my questions.
I had a minimum of one hour to think about it before making my decision.

I freely agree to participate in this study under the conditions specified in this document: ☐ **Yes** ☐ **No**

My agreement was also requested for a placental sample for biological analysis.
I give freely my permission for the analysis of the placenta:
☐ **Yes** ☐ **No**

I accept that any new information becoming available during the study and that may have implications for me will be provided by the investigating doctor:
☐ **Yes** ☐ **No**

- ✓ I understand that my data and samples will not be used for any other purpose than this research.
- ✓ I agree to participate in this research under the conditions specified in the attached information form.
- ✓ I remain free to return to my decision at any time and not to participate in the study. I will then inform the investigator.
- ✓ No longer participating in this research will not affect my relationship with my doctors and will not call into question the quality of future care.
- ✓ I have been informed that in accordance with the regulation on clinical studies, the Committee for the Protection of Persons East III has given a favorable opinion for the realization of this study dated 05 / April / 2016 and the ANSM has given its authorization for the realization of this study dated 21 / June / 2016

- ✓ I have also been informed that in accordance with the law in force, an insurance contract has been taken out by the research sponsor.

All data concerning me, including my medical file, will remain confidential. I authorize their consultation only by the persons who collaborate in the research, the persons charged by the sponsor to control the quality of the study as well as by a representative of the health authorities.

- ➢ I agree that the data recorded during this research may be the subject of a computerized treatment by the promoter or on his behalf.

I was informed of the purpose of the data processing (I was told what the data would be used for) and the recipients of this data. I have noted that my rights of access, rectification, deletion, limitation and opposition provided by the General Data Protection Regulation (GDPR) are exercised at any time by the Investigator who follow me as part of the research and who knows my identity. I confirm being affiliated to a social security scheme.

- ➢ I give my consent to participate in this research.
- ➢ I may at any time request any additional information from the investigator.
- ➢ My consent does not relieve the investigator and sponsor of all of their responsibilities and I retain all my rights guaranteed by law.
- ➢ I understand that I have the right to be informed of the overall results of this research at the end of this research. They can be communicated to me by mail (or during a follow-up consultation) if I wish.

TO BE COMPLETED BY THE PATIENT	
Date : …… Signature of the patient	
TO BE COMPLETED BY THE INVESTIGATOR DOCTOR	
I, the undersigned Doctor ..... (full name in capital letters) confirms that she has fully explained to the patient the purpose and modalities of this study as well as its potential risks. I undertake to enforce the terms of this consent form, reconciling respect for individual rights and freedoms with the requirements of scientific work. Phone number of the investigator: ……..	
Signature of the investigateur :	Date : ……..

Done in two copies, one of which will be kept by the investigator and the other given to the patient.

PATIENT CONSENT FORM

HOPE - complementary genetic study from placental samples

The details concerning this interventional research were communicated to you by the investigator verbally and were given to you in writing in a specific information document.

After reading this document and after asking the investigator all useful questions, if you agree to participate in this research, please complete the consent form below.

Title of the study : Assessment of uteroplacental vascularisation in early first-trimester pregnancy with contrast-enhanced ultrasound and 3D power Doppler angiography: protocol for a multicentre prospective study (HOPE).

SPONSOR : Centre Hospitalier Régional (CHR) de Metz-Thionville

CHR-METZ-THIONVILLE

Hôpital de Mercy 1, Allée du Château - CS 45001

57085 Metz Cedex 03.

By signing this consent form, I confirm the following:

• I understood the purpose and the modalities of this research, which were fully explained to me.

• I received the information document specific to this research and I had the opportunity to study each page carefully.

• I had a reflexion period before making my decision.

• I am compulsorily affiliated to a social security scheme.

• I declare that I am not placed under a system of legal protection of the adults (safeguard of justice, curatorship or trusteeship) and currently aimed by a proceeding tending for this purpose.

I was clearly informed:

- That I am free to accept or refuse to participate, and I am free to stop my participation in the course of research at any time. This will not influence the quality of care that will be provided to me.

- The purpose of the treatment (I was told what the data would be used for) and the recipients of this data. I have noted that my right of access, rectification and opposition provided by the law of 6 January 1978 relating to computers, files and freedoms is exercised at any time by the investigator who follows in the course of research and who knows my identity
- All data concerning me, including my medical file, will remain confidential and may be consulted by authorized persons (detailed in the attached information document) subject to professional secrecy.
- Placental data and samples may be transmitted to other national or international teams (outside the European Union) in the context of research collaborations, in a form that will not allow my direct or indirect identification.
- The placental samples can be preserved and reused in the same theme.
- An analysis of my genetic characteristics may be performed from my biological samples (as part of this research and / or for future research). This is not meant to identify or re-identify me based on my genetic characteristics.
- That I will be notified by the investigator in case of "diagnosis of a serious genetic abnormality" found during a genetic analysis: ☐ **Oui** ☐ **Non**

After having discussed and having obtained the answer to all my questions, I accept freely and voluntarily, under the conditions specified in the attached information document, to participate in the research that is proposed to me.

My consent does not relieve the investigator and sponsor of all of their responsibilities and I retain all my rights guaranteed by law.

I understand that I have the right to be informed of the overall results of this research at the end of this research. They can be communicated to me by mail (or during a follow-up consultation) if I wish.

Done in two copies, one of which will be given to me, the 2nd will be kept by the investigator.

TO BE COMPLETED BY THE PATIENT
Date: ___ / ___ / ___ Last name ……..First Name :…….. Signature of the person suitable for research:

TO BE COMPLETED BY THE INVESTIGATOR DOCTOR
Date: ___ / ___ / ___ Last name ……First Name :…… I certify that the information requirements of the person who is willing to search for and obtain his free and informed consent have been met in accordance with the regulations in force. Signature of the Investigator:

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
page 1 Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
p 9 Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
p11 Protocol version	3	Date and version identifier
p 11 Funding	4	Sources and types of financial, material, and other support
p 1 Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
p 11	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
p3-4 Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
p4 Objectives	7	Specific objectives or hypotheses
p4 Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis			
p6-7-15	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
p6-7	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
p7	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring			
p8	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.