

PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (<http://bmjopen.bmj.com/site/about/resources/checklist.pdf>) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	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).
AUTHORS	Bertholdt, Charline; ESZTO, Marie-Laure; TOURNIER, Mathilde; HOSSU, Gabriela; MELLOUKI, Naoual; CHERIFI, Aboubaker; MOREL, Olivier

VERSION 1 – REVIEW

REVIEWER	Giuseppe Rizzo Università di Roma Tor Vergata Division of Maternal Fetal Medicine Ospedale Cristo Re Roma Italy
REVIEW RETURNED	05-Apr-2019

GENERAL COMMENTS	<p>In this study Author will measure placental blood flow in the first trimester by 3D power Doppler and with a contrast media in pregnancies scheduled for termination of pregnancy. The subject is of interest and I would like to congratulate with Authors. My comments are as follows</p> <ol style="list-style-type: none">1)there is still concern in using 3D power Dopple rin the first trimester in continuing pregnancy, So data obtained from this study will be likely limited in clinical practice to recordings obtained after 11 weeks.2)standardization of setting the equipments for 3D flow assessment is of paramount importance particularly in multicentre trials. This should be included3)I will add the collection of data on placental position, depth from the maternal abdomen and volume4)concerning placenta analysis I will suggest to perform also an histomorfometric study (see Rizzo et al J Matern Fetal Neonatal Med. 2011 Feb;24(2):253-7). I f not possible at least discuss as a limitation5)the list of references should be updated
-------------------------	---

REVIEWER	Jamie Lo Oregon Health & Science University
REVIEW RETURNED	16-Apr-2019

GENERAL COMMENTS	<p>This is an important study confirming exploratory work initially published by Roberts et al. in a larger cohort and also incorporating 3D power Doppler.</p> <p>Authors plan to collect placental tissue for further analyses. Recommend considering at the minimum four quadrant sampling of the placenta to achieve representative placental sampling given prior studies from Frias et al. have demonstrated placental blood flow and histologic differences between placental cotyledons.</p>
-------------------------	--

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

Reviewer Name: Giuseppe Rizzo

Institution and Country: Università di Roma Tor Vergata

Division of Maternal Fetal Medicine

Ospedale Cristo Re

Roma Italy

Please state any competing interests or state 'None declared': none declared

Please leave your comments for the authors below

In this study Author will measure placental blood flow in the first trimester by 3D power Doppler and with a contrast media in pregnancies scheduled for termination of pregnancy. The subject is of interest and I would like to congratulate with Authors.

Thank you very much for this very positive comment

My comments are as follows

1)there is still concern in using 3D power Doppler in the first trimester in continuing pregnancy, So data obtained from this study will be likely limited in clinical practice to recordings obtained after 11 weeks.

We agree with this remark.

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.

We have added this information in the discussion section. (page 11)

2)standardization of setting the equipments for 3D flow assessment is of paramount importance particularly in multicentre trials. This should be included

The settings are standardized between the two centers.

The equipment used for the acquisitions, 3D and CEUS, are also the same because there is currently no data on the reproducibility of the measurements between different equipment and we wanted to overcome a bias. We are currently evaluating this reproducibility in another study (NCT03342014). We have added this information in the manuscript.

(Page 5: Settings and equipments are standardized between the two centers)

3)I will add the collection of data on placental position, depth from the maternal abdomen and volume. The collection of these data is planned and part of these data is mentioned in the manuscript (page 5 US acquisition).

The list of these data has been added in Table 2 in order to clarify. (Page 15)

4)concerning placenta analysis I will suggest to perform also an histomorfometric study (see Rizzo et al J Matern Fetal Neonatal Med. 2011 Feb;24(2):253-7). If not possible at least discuss as a limitation

Thanks for this very interesting remark. The villi collection, in paraffin block as mentioned in the study protocol, would allow to perform an histomorfometric analysis. We will consider the possibility of doing this during data processing.

We have added this point in the discussion part.

5)the list of references should be updated

Sorry for this mistake, we have updated the list of references.

Reviewer: 2

Reviewer Name: Jamie Lo

Institution and Country: Oregon Health & Science University

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

This is an important study confirming exploratory work initially published by Roberts et al. in a larger cohort and also incorporating 3D power Doppler.

Authors plan to collect placental tissue for further analyses. Recommend considering at the minimum four quadrant sampling of the placenta to achieve representative placental sampling given prior studies from Frias et al. have demonstrated placental blood flow and histologic differences between placental cotyledons.

The collection of trophoblasts is actually planned. However, quadrant analysis is difficult to envisage because the trophoblast is collected by surgical dilatation and evacuation. However, the histological analysis consists of the collection of all the trophoblastic villi what will be, in our opinion, representative of the whole placenta. The only limitation is the impossibility of spatial localization of the different samples due to the collection mode of the trophoblast, mainly in the form of fragments. We have added these precisions in the manuscript.

(Page 5-6: The third portion will be fixed in formalin, diluted to 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 a representative sampling of the placenta. However, it will be not possible to perform a spatial localization of the villi because placenta is surgically collected and as multiple parts.)

VERSION 2 – REVIEW

REVIEWER	Giuseppe Rizzo Università Roma Tor Vergata Italy
REVIEW RETURNED	19-Jun-2019
GENERAL COMMENTS	nicely reviewed