Periconceptional maternal conditions and virtual reality ultrasound markers of early placental health

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Thesis, Erasmus MC University Medical Centre, Rotterdam, The Netherlands

Periconceptionele maternale kenmerken en echoscopische markers van vroege placentaire gezondheid in virtuele realiteit

Periconceptional Maternal Conditions and
Virtual Reality Ultrasound Markers of Early Placental Health

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CHAPTER 1

General introduction

Modified from: Reijnders IF, Mulders AGMGJ, Koster MPH. Placental development and function in women with a history of placenta-related complications: a systematic review. Acta Obstet Gynecol Scand 2018;97(3):248-257.

Every year over 7 million women worldwide develop placenta-related complications, such as pregnancy-induced hypertension (PIH), preeclampsia (PE), fetal growth restriction (FGR) and preterm birth (PTB), that are also associated with increased risks of non-communicable diseases later in life for both mother and child[1-5]. Because these complications are largely due to abnormal placental development in the first trimester of pregnancy, research and clinical care should be focused on the periconception period, a window defined as 14 weeks prior to conception up to at least 10 weeks thereafter[4].

Placental (patho)physiology

The placenta serves as an interface between the mother, embryo and fetus. During the often neglected periconceptional period in life, the endometrium becomes receptive for implantation and thereafter endometrial decidualization takes place, which triggers trophoblast invasion and subsequent placental development[6]. This requires complex interactions between hormones, nutrients, growth factors and endometrial genes[7,8]. In the first weeks of pregnancy, human placentation is characterized by remodeling of the uterine circulation, in particular of the spiral arteries. As a result of upregulation of maternal inflammatory responses and circulatory changes[9,10], remodeling optimizes maternal blood distribution through a low-resistance uterine vascular network into the placental intervillous chamber[11]. Up to around 9 weeks of gestation, extravillous trophoblast plugs in the spiral arteries limit maternal blood entry into these intervillous chambers. These plugs gradually disintegrate thereafter, resulting in the onset of the maternal-to-fetal circulation[12]. An imbalance in this delicate process can contribute to placenta-related complications to emerge in later pregnancy stages[11-13]. Maternal endothelial dysfunction as an excessive response to placental development, is considered a key factor in placenta-related complications, triggered by an excessive maternal response to placental development.

The process of remodeling results in transformation of the uterine circulation for optimal placental development. These vascular changes, accompanied by alterations in circulating pro-angiogenic and anti-angiogenic factors, demand an intense effort of a woman's vascular capacity and as such pregnancy is considered a stress test for maternal cardiovascular health[14]. Women failing this stress test are not only at risk for developing placenta-related complications in their current pregnancy, also, the risk for recurrence of such complications in future pregnancies is increased due to underlying cardiovascular risk factors that further predispose to endothelial dysfunction[15]. Moreover, unhealthy lifestyle factors, such as smoking, alcohol use, malnutrition, and obesity influence the maternal cardiovascular condition, and as such can affect early placentation as well[16,17] **(Figure 1)**.

Markers of placental health

Unfortunately, assessment of placental health, i.e. placental development and function, during pregnancy remains challenging. Tools for *in vivo* assessment of placental health are not yet available. In recent years though, several placental markers, which can be measured already

Figure 1. Periconceptional maternal conditions and lifestyle and the impact on placental health, pregnancy course and outcome, and health of mothers and offspring during the life course
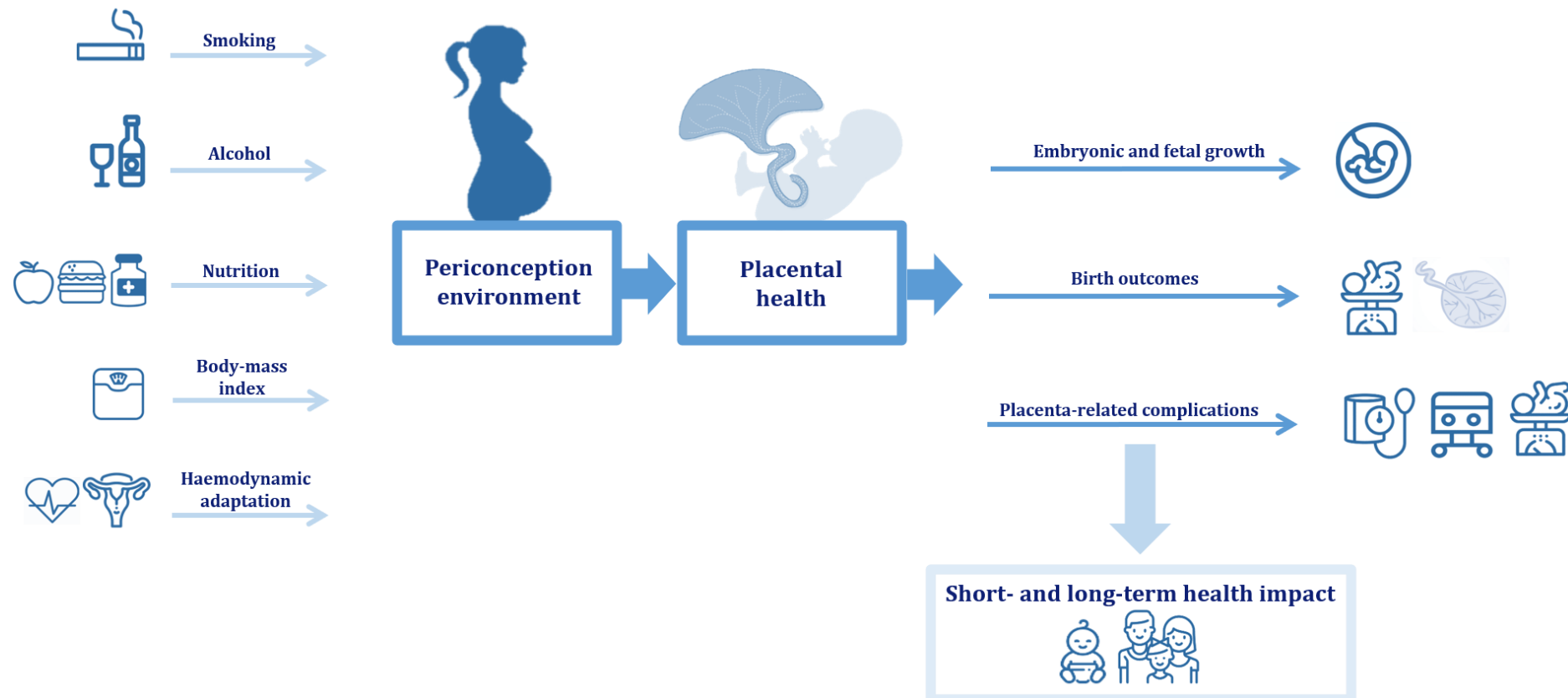
early in pregnancy, have been described[18-21]. In general, such markers are products derived from processes involved in trophoblast invasion or placental neovascularization (e.g., serum biomarkers) or maternal and/or placental (vascular) characteristics (e.g., ultrasound measurements). Furthermore, following miscarriage or delivery, placental histological features can provide information on placental tissue composition and its functionality[22].

Biochemically, abnormal placentation in early pregnancy can be characterized by excessive release of anti-angiogenic factors such as soluble Fms-like tyrosine kinase 1 (sFlt-1) and cytokines. Anti-angiogenic factors inactivate the pro-angiogenic factors vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), resulting in vascular dysfunction. The use of the sFlt-1/PlGF ratio has been proposed as a tool to screen for placental function and prognosis of PE[25-27]. Recently, it was suggested that the balance between pro- and anti-angiogenic factors is impacted by human chorionic gonadotropin (hCG)[28]. Prediction of fetal and neonatal complications using the sFlt-1/PlGF ratio is more difficult[29]. Postpartum, placental histological features like inflammatory signs or vascular abnormalities can give some information on placental tissue pathology, though the assessment of functionality is limited[22]. As placentation is a complex process, prediction models using a combination of markers (profiles) are preferred over the use of single placental markers[30-34].

Most familiar amongst ultrasound markers for non-invasive evaluation of the utero-placental circulation is the assessment of Uterine Artery (UtA) Doppler flow. Although its clinical applicability remains a topic for discussion, it is suggested that restricted UtA Doppler flow can identify women at risk for PE and FGR who subsequently qualify for preventative measures such as aspirin or intensive fetal surveillance[23,35,36]. The current state-of-the-art technology for evaluation of *in utero* placental vasculature morphology is three-dimensional power Doppler (3D PD) ultrasound[37]. 3D ultrasound imaging to assess placental health non-invasively is mainly used for quantification of placental volume or vascularization using the Virtual Organ Computer-aided AnaLysis (VOCAL) tool[24,38-41]. VOCAL uses two-dimensional (2D) planes, despite availability of 3D volumetric data. Consequently, the third dimension, enabling depth perception, is not used[42].

At the Erasmus MC, we have developed a novel, innovative application, called V-Scope, that displays volumetric ultrasound datasets as holograms, using the Barco I-Space CAVE™-like virtual reality (VR) system (Barco NV, Belgium)[40,42]. Recently, a VR desktop system, based on technical principles of the I-Space, was developed to enable clinical implementation of VR[43] **(Figure 2)**. So far, studies using VR showed accurate and reproducible embryonic and brain development measurements in early pregnancy and utero-placental measurements in the late first trimester of pregnancy[44-47].

Figure 2. Virtual Reality Barco I-space system (left) and virtual reality desktop (right), visualizing a complete utero-placental vascular volume at 12 weeks of gestation
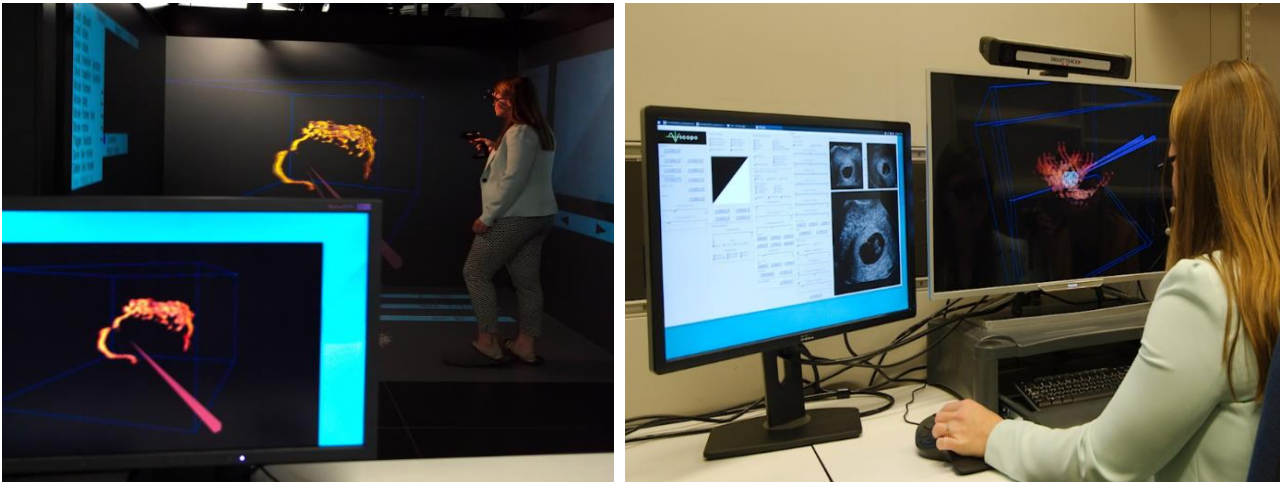
Rationale

From this background it is clear that there is a need to develop feasible and reliable non-invasive markers to assess placental health for the early detection of adverse placentation. Such markers will contribute to improved patient friendliness, feasibility of longitudinal assessment of placental health and investigation of associations with periconceptional maternal conditions, pregnancy course and outcome[48,49]. When usability and validity is shown, the diagnostic and predictive value of these placental markers for the identification of pregnancies at increased risk of placenta-related complications has to be demonstrated. Ultimately, these new markers will contribute to better clinical care and prevention of placenta-related complications, and health of mothers and offspring during the life course.

Main aim & outline of this thesis

The main aim of this thesis is to study the first-trimester trajectories of non-invasive placental markers and the associations with periconceptional maternal conditions and early placental and embryonic and fetal outcome.

The main objectives are as follows:
1. To establish first-trimester non-invasive markers of placental health and investigate the impact of periconceptional maternal conditions and lifestyle **(Chapter 2 and 3)**.
2. To study associations between early placental development and embryonic and fetal growth, and birth outcome **(Chapter 4 and 6)**.
3. To determine the impact of first-trimester maternal haemodynamic adaptation on placental, embryonic and fetal development as well as birth outcome **(Chapter 5)**.

The General Discussion is presented in **Chapter 7** and the results of this thesis are summarized in **The Addendum**.

Setting

Data for this thesis was obtained from a prospective cohort study (Virtual Placenta Study, 2017-2018) embedded in the ongoing Rotterdam Periconceptional Cohort (Predict Study), since 2009 conducted at the Department of Obstetrics and Gynaecology, Erasmus MC University Medical Center, Rotterdam.

Rotterdam Periconceptional Cohort
The Rotterdam Periconceptional Cohort is a prospective cohort study conducted in the high-risk population of a tertiary hospital, with a focus on determinants of periconceptional health, pregnancy course and outcome and underlying epigenetic mechanisms[50,51]. Pregnant women are recruited before and in the early first trimester of pregnancy and followed up until one year after delivery. During pregnancy, transvaginal three-dimensional ultrasound scans are performed at 7, 9 and 11 weeks of gestation. At study entry, maternal anthropometrics are recorded (body-mass index, blood pressure and waist-hip ratio), a blood withdrawal is taken and data on maternal characteristics, medical and obstetrical history, data on dietary patterns and lifestyle are obtained from self-reported questionnaires, verified by a researcher. Data on pregnancy course and outcome are also obtained from self-reported questionnaires and validated by medical records. At birth, umbilical cord blood withdrawals are taken. Postpartum, in a subset of women, pregnancy outcomes are monitored by questionnaires and medical records.

Virtual Placenta Study
Between January 2017 until March 2018, women were invited to participate in the Virtual Placenta Study before 10 weeks of gestational age (GA). In addition to the Rotterdam Periconceptional Cohort study, further visits were scheduled at 13, 22 and 32 weeks of gestation. During the first-trimester visits (7, 9 and 11 weeks), participants underwent 3D ultrasounds including power Doppler recordings of the utero-placental circulation. At all study visits (7, 9, 11, 13, 22 and 32 weeks of gestation), bilateral uterine artery blood flow was recorded by pulsed wave Doppler ultrasound. At all visits, maternal anthropometrics were recorded and blood withdrawals were taken. Postpartum, placental histology and/or placental perfusion was assessed in a subset of women **(Figure 3)**.

Figure 3. Study design and timeline of the Rotterdam Periconception Cohort (blue pictograms) and Virtual Placenta Study (red pictograms)
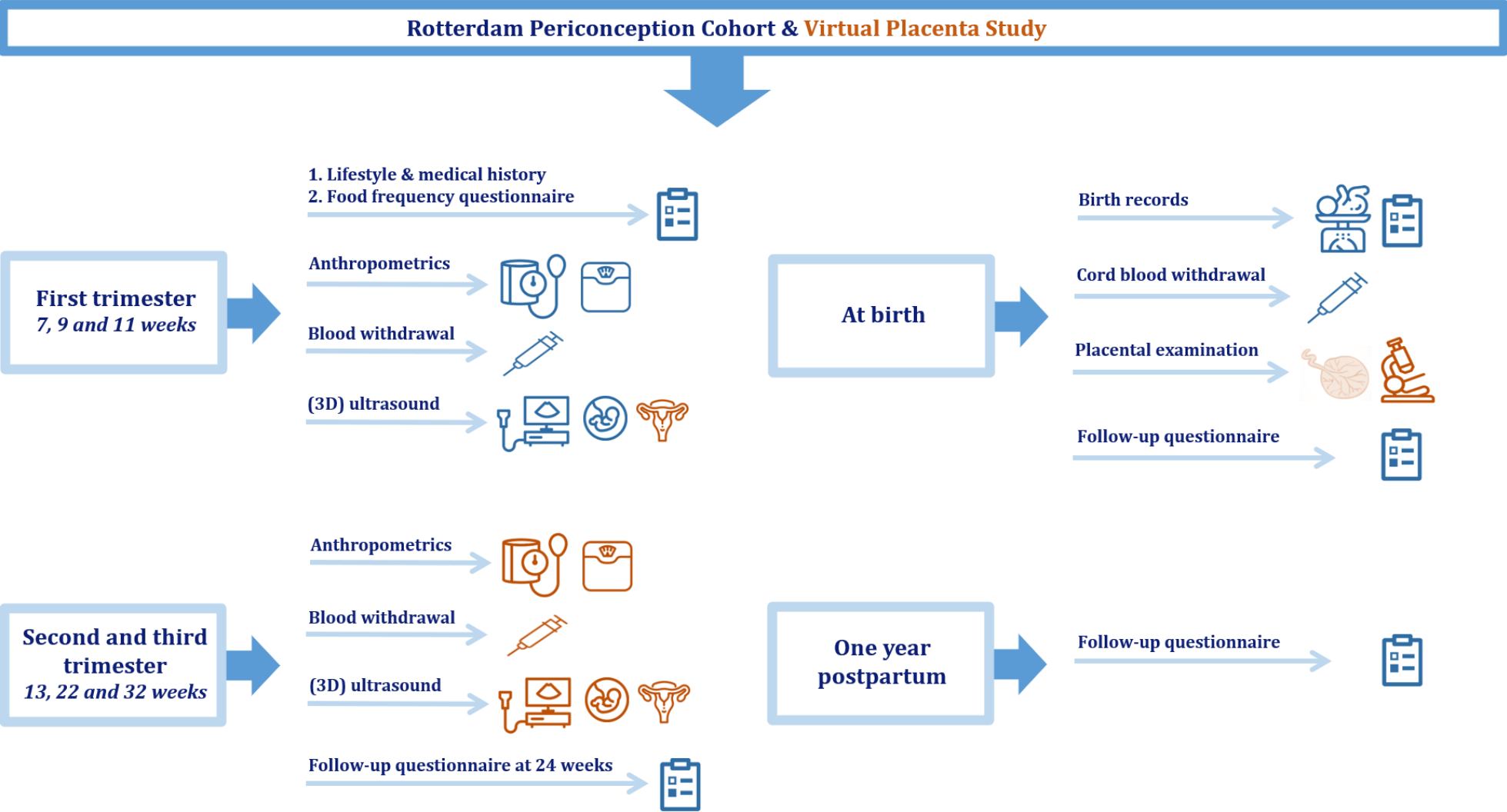
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CHAPTER 2

The impact of periconceptional maternal lifestyle on clinical features and biomarkers of placental development and function: a systematic review

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ABSTRACT

Background: Worldwide, placenta-related complications contribute to adverse pregnancy outcomes, such as pre-eclampsia, fetal growth restriction and preterm birth, with implications for future health of mothers and offspring. The placenta develops in the periconception period and forms the interface between mother, embryo and fetus. Unhealthy periconceptional maternal lifestyle, such as smoking, alcohol and under- and overnutrition, can detrimentally influence placental development and function.

Objective and rationale: The impact of maternal lifestyle on placental health is largely unknown. Therefore, we aim to summarize the evidence of the impact of periconceptional maternal lifestyle on clinical features and biomarkers of placental development and function throughout pregnancy.

Search methods: A comprehensive search in Medline, Embase, Pubmed, The Cochrane Library Web of Science and Google Scholar was conducted. The search strategy included keywords related to the maternal lifestyles smoking, alcohol, caffeine, nutrition (including folic acid supplement intake) and body weight. For placental markers throughout pregnancy keywords related to ultrasound imaging, serum biomarkers and histological characteristics were used. We included randomized controlled trials and observational studies published between 2000-2017 (March) and restricted the analysis to singleton pregnancies and maternal periconceptional lifestyle. Methodological quality was scored using the ErasmusAGE tool. A protocol of this systematic review has been registered in PROSPERO International prospective register of systematic reviews (PROSPERO 2016:CRD42016045596, available from http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016045596).

Outcomes: Of 2,593 unique citations found, 82 studies were included. The median quality score was 5 (range 0-10). It revealed that maternal smoking was associated with lower first-trimester placental vascularization flow indices, and higher second- and third-trimester resistance of the uterine and umbilical arteries and lower resistance of the middle cerebral artery. Although a negative impact of smoking on placental weight was expected, this was less clear. Alcohol use was associated with a lower placental weight. One study described higher second- and third-trimester placental growth factor (PlGF) levels after periconceptional alcohol use. None of the studies looked at caffeine intake. Adequate nutrition in the first trimester, periconceptional folic acid supplement intake and strong adherence to a Mediterranean diet, were all associated with a lower resistance of the uterine and umbilical arteries in the second and third trimester. A low caloric intake resulted in a lower placental weight, length, breadth, thickness, area and volume. Higher maternal body weight was associated with a larger placenta measured by ultrasound in the second and third trimester of pregnancy or weighed at birth. In addition, higher maternal body weight was associated with decreased PlGF-levels.

Wider implications: Evidence of the impact of periconceptional maternal lifestyle on placental health was demonstrated. However, due to poorly defined lifestyle exposures and time windows of investigation, unstandardized measurements of placenta-related outcomes and small sample sizes of the included studies, a cautious interpretation of the effect estimates is indicated. We suggest that future research should focus more on physiological consequences of (un)healthy

lifestyle during the critical periconception window. Moreover, we foresee that new evidence will support the development of lifestyle interventions to improve the health of mothers and their offspring from the earliest moment in life.

INTRODUCTION

Worldwide, placenta-related pregnancy complications, such as pregnancy-induced hypertension (PIH), pre-eclampsia (PE), fetal growth restriction (FGR) and preterm birth (PTB), significantly contribute to adverse outcome of pregnancy in the mother and neonate[1]. These adverse short-term health outcomes are also associated with increased risks of non-communicable diseases in later life[2-4]. Epigenetics is hypothesized to be one of the underlying mechanisms of these associations, in which placental development and function are strongly involved[5].

The most important development of the placenta takes place in the periconception period, a window defined as 14 weeks before conception up to at least 10 weeks thereafter, and serves as an interface between mother, embryo and fetus[6]. During this often neglected period in life, the endometrium becomes receptive for implantation and thereafter the endometrium is decidualized, which triggers the start of trophoblast invasion and subsequent placental development[7]. In this process, spiral arteries are transformed into low-resistance vessels resulting from maternal circulatory changes and upregulation of inflammatory responses[8,9]. Successful placental development strongly depends amongst other pathways on the capacity of haemodynamic adaptation[10]. Moreover, unhealthy lifestyle factors, such as smoking, alcohol use, malnutrition and obesity influence maternal cardiovascular condition, and as such can affect early placentation resulting in increased risks for adverse pregnancy outcomes[11,12].

Unhealthy maternal lifestyles which also includes, over- and undernutrition, are modifiable conditions that are highly prevalent also during pregnancy and associated with the occurrence of placenta-related PE, FGR, and PTB[13-15]. In our previous studies we found significant associations between periconceptional maternal smoking, alcohol intake, healthy dietary patterns and embryonic growth trajectories[16-18]. We therefore hypothesize that these maternal conditions can also interfere with placental development and function from the periconception period onwards[19].

It is largely unknown which pathways are involved and to what extent periconceptional lifestyle influences first-trimester placental development and function[19,20]. Maternal smoking is the most studied exposure with a prevalence between 10 and 40% at any time during pregnancy and shows significant associations with FGR and PTB[11,21]. One of the mechanisms involving the placenta is based on the evidence that nicotine acts as a pro-angiogenic factor and thereby supports migration, proliferation and in vitro vasculogenesis of endothelial progenitor cells[22,23]. In addition, the relative hypoxia caused by smoking results in excessive oxidative stress that can induce alterations of placental villi, thereby impairing placental development and function[11,24].

A substantial number of women report periconceptional social alcohol use. The prevalence varies in women during pregnancy from 40% in the United Kingdom to 72% in Australia[25]. A

previous review has shown a short-term negative impact of periconceptional alcohol intake on placental blood flow parameters[26]. Moreover, long-term effects of alcohol were reported to change placental villous tissue, which coincides with FGR[26].

Maternal caffeine intake is also a modifiable lifestyle contributing to placental disorders. This substance has a similar structure as DNA purine molecules and therefore can result in an inhibition of cell division and metabolism. Additionally, increased adrenal medullar catecholamine release following caffeine intake is associated with uteroplacental circulation vasoconstriction. It is also known that caffeine easily diffuses through placental tissues from mother to embryo/fetus, which may explain the negative association with birth weight[27].

Under- or overnutrition, negatively impact several pathways, of which one-carbon (1-C) metabolism is most prominent during the periconception period[6]. 1-C metabolism is essential for DNA synthesis, repair, methylation and phospholipid and protein synthesis. Disturbances of this metabolism result in an overwhelming production of reactive oxygen species, i.e., excessive oxidative stress, which can have a detrimental impact on reproduction and presumably also on placentation[28]. Substrates or cofactors in 1-C metabolism are nutrients and vitamins, such as methionine, folate, riboflavin, pyridoxin and cobalamin[6]. Overweight and obesity are often the consequence of an accumulation of unhealthy lifestyles. During pregnancy both phenotypes can lead to an exaggerated systemic and placental inflammatory response, characterized by accumulation of macrophages and production of pro-inflammatory mediators[29]. These pregnancies are more often complicated by maternal morbidity, such as hypertensive disorders of pregnancy and gestational diabetes, conditions that can affect growth and development of the embryo and fetus[30,31]. It has been hypothesized that a higher maternal body mass index (BMI) not only modifies placental function by exaggerated inflammation, but also by altering placental development[32]. Obesity also affects angiogenesis resulting in placental vascular abnormalities or epigenetic placental alterations[33,34].

In vivo investigation of placentation in human pregnancy is currently not possible. However, several markers of placental development and function have been described as proxies[35-38]. A distinction is made between markers that reflect maternal and utero-placental vascular function, i.e., ultrasound measurements, such as uterine or umbilical artery Doppler flow assessment, and serum biomarkers that are expressed during trophoblast invasion or placental neovascularization.

A well-studied ultrasound marker for non-invasive evaluation of the uteroplacental circulation is Uterine Artery (UtA) Doppler flow. It is suggested that restricted UtA Doppler flow can identify women at risk for PE and FGR who could benefit from preventative measures such as aspirin or regular fetal monitoring[39]. New ultrasound markers to assess placental development and function are measurements of placental volume and vascularization indices using three-dimensional ultrasound[36,40].

Biochemically, abnormal placentation in early pregnancy can be characterized by excessive release of anti-angiogenic factors such as soluble Fms-like tyrosine kinase 1 (sFlt-1) and cytokines. The anti-angiogenic factors inactivate the pro-angiogenic factors vascular endothelial growth factor (VEGF) and PlGF, resulting in vascular dysfunction. The use of the sFlt-1/PlGF ratio has been

proposed most commonly as a tool to screen for placental function and prognosis of PE[41-43]. Prediction of fetal and neonatal complications using the sFlt-1/PlGF ratio is more challenging[44].

Postpartum placental histological features like inflammatory signs or vascular abnormalities can give some information on placental tissue pathology, though the assessment of functionality is limited[45]. As placentation is a complex process, prediction models using a combination of markers (profiles) are preferred over the use of single placental markers[46-50].

Since unhealthy maternal lifestyles are highly prevalent before and during pregnancy and are linked to cardiovascular risk factors, we here aim to summarize the evidence of the impact of periconceptional maternal lifestyle on markers of placental development and function throughout pregnancy. More specifically in this review, we focus on clinical features and biomarkers with clinical relevance, such as ultrasound imaging, serum concentrations of biomarkers and placental gross and histopathological features.

METHODS

Sources

A search in Medline, Embase, Pubmed, The Cochrane Library (Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials (CENTRAL), Cochrane Methodology Register), Web of Science and Google Scholar was conducted. Keywords included in our search strategy were related to the periconceptional maternal lifestyles smoking, alcohol and caffeine intake, nutrition (including folic acid supplement intake) and body weight, which were combined using the Boolean operator 'or' **(Supplemental File 1)**. For markers of placental development and function, keywords related to the structural and functional placental parameters ultrasound imaging, serum biomarkers and histological characteristics were used. We searched reference lists from included studies and systematic reviews to include relevant remaining articles. A protocol of this systematic review was registered in PROSPERO International prospective register of systematic reviews (PROSPERO 2016:CRD42016045596, available from http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016045596).

Eligibility

To best reflect current daily practice, articles published in English between January 2000 and March 2017 were eligible for inclusion. Eligible studies required inclusion of women, with a singleton pregnancy at any gestation, in whom at least one of the lifestyles in the periconceptional period was studied. To acquire the highest level of evidence, we selected randomized trials and observational studies, including cohort- and case-control studies. Letters to the editor, conference abstracts, editorials and case reports were not eligible. Systematic reviews were also excluded, but their relevant references were checked first.

Authors (I.R., A.M. and M.v.d.W.) independently screened titles and abstracts of studies that resulted from the search strategy to identify eligible articles for full-text screening. Then, two

reviewers (I.R. and M.v.d.W.) independently assessed the eligibility of the full-text articles. Discussion with a third reviewer (A.M.) resolved any disagreement about the in- or exclusion of a full-text article.

Key information about the included studies was collected in a standardized data extraction form. Extracted data included information on study design, study population, methodology (inclusion period, exclusion criteria, statistical methods and adjustment for confounders), the studied exposure (lifestyle), the outcome of placental development or function, and the study results. If data were incomplete or missing, study authors were requested to provide their data.

I.R. and M.v.d.W. independently assessed the quality of included studies with the predefined ErasmusAGE quality score **(Supplemental File 2)**, which, based on previously published scoring systems, has been developed by the Rotterdam Intergenerational Ageing Research Center (www.erasmusage.com) at Erasmus MC (National Collaborating Centre for Methods and Tools, 2008)[51]. For appraisal of randomized trials and observational studies, the score is composed of five items: study design, study size, description of methods to assess the lifestyle of interest, markers of placental development and function, and correction for confounders in data analysis. We chose specific quantitative limits for the items; what we considered an appropriate study size, for example, was based on literature and opinions of the research group members. Each item was allocated 0, 1 or 2 points, resulting in a total score from 0 to 10, with 10 representing the highest quality.

Initially, baseline study characteristics of all included studies were extracted. Numerous markers of placental development and function were retrieved. As commonly used in literature, markers of placental development and functions were categorized as ultrasound imaging, serum biomarkers and histological characteristics. Ultrasound markers were either static (placental measures or other observations) or dynamic features (Doppler flow velocimetry). Serum biomarkers were categorized as either proteomics, metabolomics or genomics and then subcategorized as markers of vascular function, inflammation, oxidative stress or miscellaneous. Metric features were related to measures of placental size at birth. Histological features were categorized as either being of maternal or fetal origin and followed by subcategories such as a sign of oxidative stress, vascular or inflammatory changes or miscellaneous.

Within the subgroups of ultrasound imaging, serum biomarkers and histology, we further detailed markers of placental development and function with known clinical applicability – not only to narrow our results, but also to generate an overview with more clinical relevance. Ultrasound imaging markers had to focus on static (placental volumes, placental grading) or dynamic (Doppler flow velocimetry of the uterine, umbilical or middle cerebral arteries) measurements. For reasons of clinical applicability, serum biomarkers were restricted to PlGF, sFlt-1, soluble endoglin (sEng), pregnancy-associated plasma protein A (PAPP-A), fetal beta human chorionic gonadotropin (fβhCG) and inhibin A. No restrictions were made for placental histological features.

Odds ratios (OR) with corresponding 95% confidence intervals (CI) were calculated for markers of placental development and function when these data were available in the original article. Number of subjects exposed to the lifestyle of interest with respect to the placental marker of

interest (1) were divided by number of subjects without exposure to that respective lifestyle or nutritional factor and the placental marker of interest (2). Next, the resulting number was divided by the number of subjects exposed to a lifestyle or nutritional factor without the placental marker of interest (3) divided by the number of subjects without the exposure to that respective lifestyle or nutritional factor as well as the placental marker of interest (4; OR = (1/2) (3/4)). When study population, definitions of the lifestyle exposure and placental outcome parameter were similar between studies, ORs were pooled. A meta-analysis was not performed due to the large heterogeneity of the definitions of periconceptional exposures and markers of placental development and function.

RESULTS

Search results
Figure 1 shows the flowchart of the selection process of studies included for systematic review*.* The initial search strategy yielded 4,811 articles, of which 2,593 unique articles remained after exclusion of duplicates. In total 2,374 articles did not fulfill the predefined eligibility criteria and were excluded after screening of titles and abstracts. Screening of full texts of the remaining 219 articles resulted in 76 studies eligible for inclusion. Cross-reference checking resulted in an additional inclusion of six articles.

Study characteristics and quality assessment
Supplemental table 1 provides an overview of the baseline characteristics and quality scores of the included studies. The studies originated from 33 different countries. Three randomized controlled trials, 23 case-control studies, 44 observational cohort studies and 12 cross-sectional design studies were included. The quality of these studies ranged from poor to good, with a quality score median of 5 (range: 2 - 10). **Supplemental tables 2a, 2b** and **2c** provide a schematic overview of the associations between periconceptional maternal lifestyle and markers of placental development and function per trimester of pregnancy**.**

   The following subsections describe the associations between periconceptional lifestyle and various placental markers throughout pregnancy. Only associations between exposures and outcomes that are relevant for daily practice or have clinical implications are described. None of the included studies addressed the impact of periconceptional caffeine intake on placental development and function. Detailed results for the markers of placental development and function in each category (ultrasound imaging, serum biomarkers, histology) are described per periconceptional maternal exposure (smoking, alcohol and caffeine intake, nutrition and body weight) in **Tables 1-4**. An overview of the calculated ORs, where available, for markers of placental development and function in association with lifestyle, is displayed in **Table 5**.

Figure 1. Flow chart of study selection
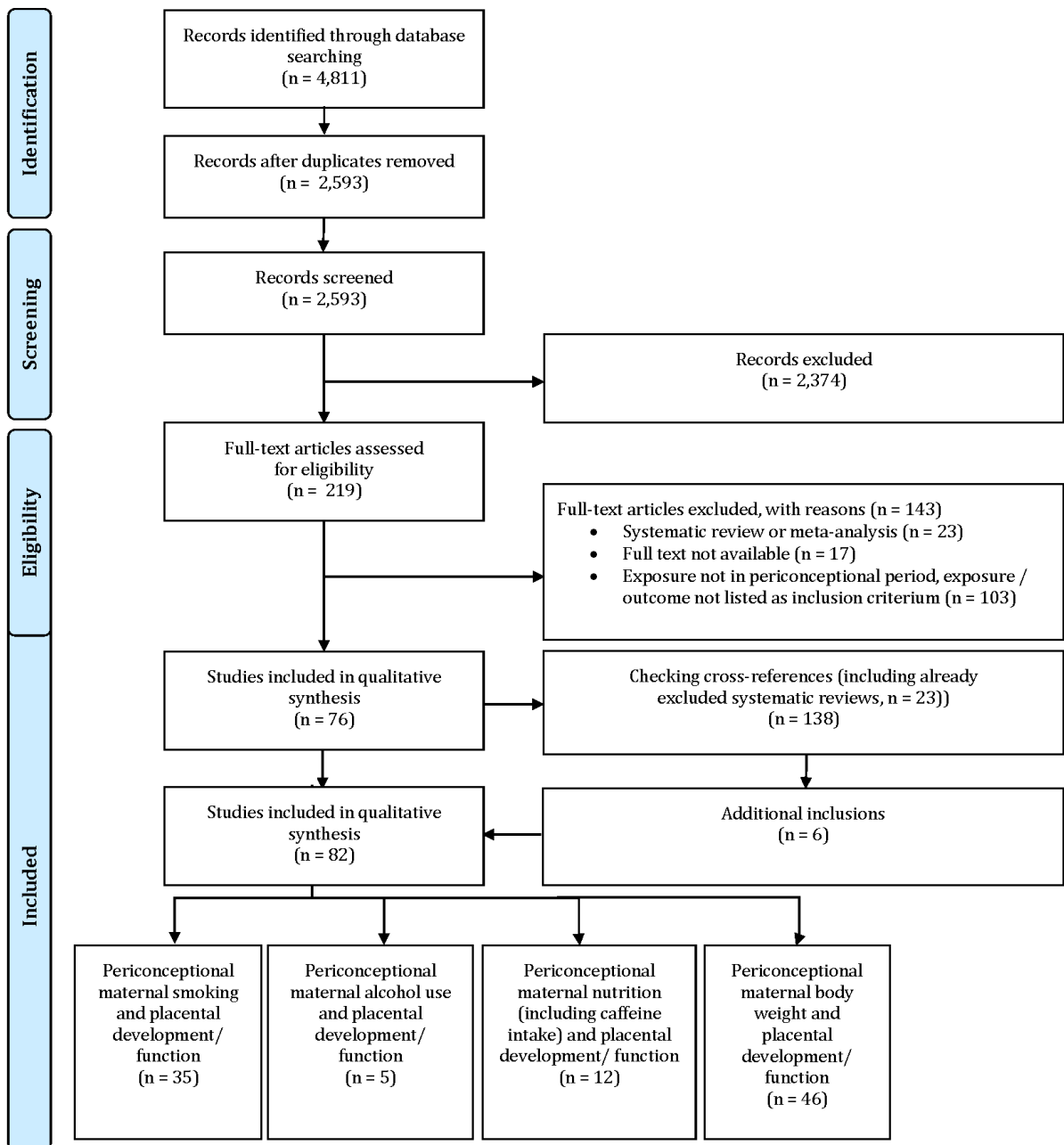
Maternal smoking

Twenty-eight studies focused on the influence of periconceptional maternal smoking on markers of placental development and function **(Table 1)**. Overall, we found a detrimental influence of maternal smoking on markers of placental development and function. In general, ultrasound studies showed lower vascularization indices[107] and increased second- and third-trimester Doppler pulsatility and resistance indices of the maternal UtA and the fetal umbilical artery (UmbA)[66,85,106]. Where available, ORs are displayed in **Table 5**. A study by Geelhoed *et al.,* (2011) identified no such significant differences for the maternal UtA pulsatility index (PI), fetal UmbA PI or fetal middle cerebral artery (MCA) PI in the third trimester of pregnancy[73]. No significant differences for late first-, second- and third-trimester placental volume (in mL and cm^3) were identified[80,107].

Associations between maternal smoking and placental serum biomarkers were found as well. Serum PlGF-levels in the second trimester of pregnancy were higher[81,97]. No differences were found for sFlt-1[81,97]. Furthermore, decreased PAPP-A-levels[63,64,80] and decreased fβhCG-levels[80,82] were found in smokers in all trimesters, except in a study by Yigiter *et al.,* (2006), that found no difference[125].

In relation to maternal smoking, the main studied feature of placental pathology was placental weight. Placental weights were decreased in periconceptional smokers[87,101,102,123]. This was further confirmed by an increased OR for lower placental weight in smokers, reported by Niu *et al.,* (2016) (OR 2.30, 95% CI 1.10, 4.84)[102]. Five other studies showed no significant difference[53,62,106,114] or a higher placental weight[96] Macdonald *et al.,* (2014) showed lower probabilities for a placental weight ratio below the 10th centile in pregnancies in late preterm (≥33 - <37 weeks) births versus term (≥37 weeks) births (OR 0.49, 95% CI 0.26, 0.95 and OR 0.65, 95% CI 0.56, 0.75 respectively), indicating more inefficient placentas (i.e. more placental tissue per fetal mass) in smokers[94].

Maternal alcohol use

Five studies focused on the impact of periconceptional maternal alcohol use on markers of placental development and function **(Table 2)**. One of these studies focused on markers of placental function in the second and third trimesters of pregnancy, showing higher PlGF-levels in women with periconceptional alcohol intake, defined as ≥8 drinks per week[121]. Three other studies investigated features of placental histology at term in women with periconceptional alcohol intake, and found decreased placental weights[62,114,123].

No significant ORs were found for periconceptional maternal alcohol use in association with markers of placental development and function.

Maternal nutrition

Twelve studies focused on the impact of periconceptional maternal nutrition on markers of placental development and function **(Table 3)**. Periconceptional micronutrient supplementation, folic acid supplement intake and adherence to a Mediterranean diet were associated with decreased mean Doppler indices of the second- and third-trimester maternal UtA RI and

PI[103,116,117]. Women with a periconceptional folic acid supplement intake and adherence to a Mediterranean diet had a lower fetal UmbA PI[116,117]. The studies on serum biomarkers PlGF and sFlt-1 which were retrieved in association with maternal nutrition were heterogeneous for the type of nutrition that was investigated.

Various nutritional factors, i.e., general dietary intake, first-trimester micronutrient supplementation and folic acid supplement intake, showed varying influences on features of placental pathology, mainly reflected in placental weight. In studies evaluating maternal dietary patterns, lower adherence to the Mediterranean diet in the first trimester of pregnancy showed lower placental weights[117], while other studies showed no associations between placental weight at birth and intake of any of the first-trimester nutrients[75,88]. Abreu *et al.,* (2016) and Moore *et al.,* (2004) observed a greater placental weight in women with respectively greater dairy intake or a higher percentage of energy derived from protein intake in the first trimester[52,98]. Additionally, placental dimensions (length, breadth, thickness, area and volume) in neonates from mothers who experienced periconceptional famine were smaller[108]. Due to the heterogeneity in methods applied and data retrieved, ORs for periconceptional nutrition in association with markers of placental development and function could not be calculated.

Maternal body weight
Thirty-three studies focused on the influence of periconceptional maternal body weight on markers of placental development and function **(Table 4)**. In general, higher periconceptional maternal body weight was associated with a larger placental volume (in mL and cm^3) on ultrasound in the second and third trimesters of pregnancy[86,113,124].

Higher periconceptional maternal body weight showed various results for markers of placental development and function. Serum PlGF-levels throughout pregnancy were either decreased[97,126] showed no difference[71]. Levels of sFlt-1 throughout pregnancy were either increased[71] or decreased[97,126].

For higher periconceptional maternal body weight in relation to features of placental pathology, we identified 18 studies showing a higher placental weight at birth[30,56,68,70,74,77,87,89,93-96,112,114,118,122,123]. Where available, ORs are displayed in **Table 5**. Three other studies showed no difference in placental weight[57,69,109] in case of higher periconceptional maternal body weight.

Moreover, a higher placenta-to-birth weight ratio was reported in studies by Huang *et al.,* (2014); Berglund *et al.,* (2016) and Mando *et al.,* (2016)[31,79,95]. Wang *et al.,* (2014) found no difference in the placenta-to-birth weight ratio in association with pre-pregnancy BMI[123].

One study, by Wallace *et al.,* (2014), addressed the impact of inter pregnancy weight change on placental weight. The association with placental weight above the 90th centile was stronger in women with an increase of more than 3 BMI points (OR 1.59, 95% CI 1.31, 1.47)[122].

Table 1. Summary of the associations between maternal periconceptional smoking and markers of placental development and function (28 studies)

Author (year)	Study design	Sample size	Exposure definition	Parameter type	Definition of marker of placental development and function	Main result(s)
Rizzo et al., 2009[107]	Prospective cohort study	80	First-trimester maternal smoking <10 cigarettes/day; 10-20 cigarettes/day; >20 cigarettes/day	Ultrasound	Late first-trimester placental volume (mL)	=
					Late first-trimester vascularization index	<
					Late first-trimester flow index	<
					Late first-trimester vascularization flow index	<
Jauniaux et al., 2013[80]	Prospective cohort study	128	First trimester maternal smoking between 10-20 cigarettes/day	Ultrasound	Late first or second trimester placental thickness (mm)	=
					Late first or second trimester basal plate surface (mm^2)	=
					Late first or second trimester placental 2D volume (cm^3)	=
Kho et al., 2009[85]	Prospective cohort study	2,459	Maternal smoking at 15±1 weeks GA (women ceased <15 weeks GA: non-smokers)	Ultrasound	Second trimester abnormal UtA RI (>90th centile)	>
					Second trimester abnormal UmbA RI (>90th centile)	>
de B Machado et al., 2011[66]	Prospective cohort study	64	Any maternal cigarette smoking from the periconceptional period up to the third trimester of pregnancy, confirmed by exhaled air carbon monoxide and urinary cotinine concentrations	Ultrasound	Third trimester UtA RI	>
					Third trimester UmbA RI	>
					Third trimester MCA RI	<
Geelhoed et al., 2011[73]	Prospective cohort study	1,120	First trimester maternal smoking; continued smoking (<5 cigarettes/day or >5 cigarettes/day)	Ultrasound	Third trimester UtA PI	=
					Third trimester UmbA PI	=
					Third trimester MCA PI	=
					Third trimester MCA PSV (cm/s)	=

Table 1, continued

Author (year)	Study design	Sample size	Exposure definition	Parameter type	Definition of marker of placental development and function	Main result(s)
Pringle *et al.*, 2005[106]	Case-control	1,484	Preconceptional maternal smoking; <10 cigarettes/day; 10-20 cigarettes/day; >20 cigarettes/day	Ultrasound	Second- and-third trimester UtA PI	=
					Second-trimester UmbA PI	=
					Third-trimester UmbA PI	>
					Placental grading according to Grannum (grade 0 normal, grade 1 random echogenic areas, grade 2 basal echogenic areas and indentations in chorionic plate, grade 3 echo-poor areas, irregular echogenic areas and deep indentations in the chorionic plate)	>(more Grannum grade 1 and 2)
Chelchowska *et al.*, 2012[63]	Prospective cohort study	60	Minimum maternal smoking of 5 cigarettes/day during pregnancy	Serum biomarker	First-trimester mean serum concentrations of PAPP-A (U/l)	<
Chełchowska *et al.*, 2016[64]	Nested case-control	150	Minimum maternal smoking of 5 cigarettes/day during pregnancy and at least 2 years before conception	Serum biomarker	Serum PAPP-A levels (IU/L) in each trimester	<
Jauniaux *et al.*, 2013[80]	Prospective cohort study	128	First trimester maternal smoking between 10-20 cigarettes/day	Serum biomarker	First- and second-trimester PAPP-A levels (MoM)	<
					First- and second-trimester fβhCG levels (MoM)	<
					First- and second-trimester inhibin A levels (MoM)	>
Kagan *et al.*, 2007[82]	Retrospective cohort study	109,263	Self-reported maternal cigarette smoking status at time of conception (number of cigarettes 1-2/day, 3-5/day, 6-10/day, 11-15/day, >15/day)	Serum biomarker	First-trimester PAPP-A levels (MoM)	<
					First-trimester fβhCG levels (MoM)	<
Yigiter *et al.*, 2006[125]	Prospective cohort study	1,275	Self-reported maternal cigarette smoking (<5/day or >5/day)	Serum biomarker	First-trimester PAPP-A levels (MoM)	<
					First-trimester fβhCG levels (MoM)	=
Jeyabalan *et al.*, 2010[81]	Prospective cohort study	993	Preconceptional maternal smoking, quit at start of pregnancy, continued smoking and smoking of >1 pack of cigarettes/day	Serum biomarker	PlGF levels (pg/ml)	>
					sFlt1 levels (ng/ml)	=
					sEng levels (ng/ml)	<

Author (year)	Study design	Sample size	Exposure definition	Parameter type	Definition of marker of placental development and function	Main result(s)
Mijal *et al.,* 2011[97]	Prospective cohort study	668	Stopped maternal smoking before enrolment (second trimester), smoking <half pack/day at enrolment, smoking ≥half pack per day at enrolment	Serum biomarker	Second-trimester PlGF levels (ng/ml)	>
					Second-trimester sFlt1 levels (pg/ml)	=
					Second-trimester sEng levels (pg/ml)	=
Macdonald *et al.,* 2014[94]	Cross-sectional	2,016	Maternal smoking during pregnancy (yes/no)	Histology	PWR <10th centile in preterm pregnancies	>
					PWR <10th centile in term pregnancies	>
					PWR >90th centile in term pregnancies	<
L'Abée *et al.,* 2011[87]	Prospective cohort study	2,947	Undefined	Histology	Placental weight (grams)	<
McNamar *et al.,* 2014[96]	Retrospective cohort study	97,600	Maternal smoking during pregnancy (yes/no)	Histology	Placental weight (grams)	>
Niu *et al.,* 2015[101]	Case-control	2,143	Average passive maternal smoking exposure during pregnancy (arithmetic mean of number of minutes per trimester)	Histology	Placental weight (grams)	<
Niu *et al.,* 2016[102]	Case-control	390	Average passive maternal smoking exposure during pregnancy (arithmetic mean of number of minutes per trimester)	Histology	Placental weight (grams)	<
Pringle *et al.,* 2005[106]	Case-control	1,484	Preconceptional maternal smoking; <10 cigarettes/day; 10-20 cigarettes/day; >20 cigarettes/day	Histology	Placental weight (grams)	=
Akbulut *et al.,* 2009[53]	Cross-sectional	92	Maternal smoking throughout pregnancy	Histology	Placental weight (grams)	=
					Chorangiosis (hyperplasia of the terminal chorionic villi = the occurence of >10 villi with >10 capillaries in 10 low-power microscopic fields of non-infarcted areas)	>
					Villous numbers (in 10 different areas)	<
					Vasculosyncytial membrane thickening (presence of trophoblast and endothelial basal membrane thickness areas in periodic acid Schiff-stained preparations)	>
Carter *et al.,* 2016[62]	Prospective cohort study	103	Undefined	Histology	Placental weight (grams)	=
					Intimal cushions	=

Table 1, continued

Author (year)	Study design	Sample size	Exposure definition	Parameter type	Definition of marker of placental development and function	Main result(s)
Tikellis *et al.,* 2012[114]	Prospective cohort study	7,945	Maternal smoking during pregnancy: never, 1-10 cigarettes/day, 11-20 cigarettes/day, >20 cigarettes/day	Histology	Placental weight (grams)	=
Wang *et al.,* 2014[123]	Prospective cohort study	7,945	Maternal smoking during pregnancy: never, 1-10 cigarettes/day, 11-20 cigarettes/day, >20 cigarettes/day	Histology	Placental weight (grams)	<
					Placenta-to-birth weight ratio	>
					Classification by visual inspection as normal, incomplete, infarcted, post mature, clots on maternal side	=
					Other placental abnormality	=
Avagliano *et al.,* 2012[55]	Cross-sectional	203	Undefined	Histology	Abnormal spiral artery modification: intact vessel wall without evidence of trophoblast invasion in at least 70% of the arterial sections	=
Bush *et al.,* 2000[61]	Prospective cohort study	92	Undefined	Histology	Maternal intervillous space volumes (in cm^3)	>
					Surface areas of fetal capillaries (in m^2)	>
					Trophoblast component in villous membrane (in μm)	>
					Fetal capillary volumes (in cm^3)	<
Genbacev *et al.,* 2000[24]	Case-control	96	First trimester maternal smoking: <10 cigarettes/day; 11-20 cigarettes/day; >20 cigarettes/day	Histology	Cytotrophoblast stem cell population	<
Gruslin *et al.,* 2001[76]	Cross-sectional	129	Undefined	Histology	First-trimester trophoblast apoptosis	>
					Third-trimester placental Xiap levels	>
					Third-trimester procaspase-3 levels	>
van Oppenraaij *et al.,* 2012[120]	Case-control	26	Maternal smoking of at least 10 cigarettes/day daily in the preconceptional period up	Histology	First-trimester villous tree vascular densities	>

BMI= body-mass index (kg/m², unless stated otherwise); cm= centimeter; fβhCG= fetal beta human chorionic gonadotropin; IU/l= international units per liter; m= meter; MCA= middle cerebral artery; μm= micro meter; mL= milliliter; mm= millimeter; MoM= multiples of the median; ng= nanogram; PAPP-A= pregnancy-associated plasma protein A; PI= pulsatility index; PlGF= placental growth factor; pg= picogram; PSV= peak systolic velocity; PWR= placental weight ratio; RI= resistance index; sFlt= soluble fms-like tyrosine kinase; sEng= soluble endoglin; U/l= units per liter; UmbA= Umbilical artery; UtA= Uterine artery

Main result in red= negative impact of periconceptional lifestyle exposure on described placental marker of development and function; main result in orange = no impact of periconceptional lifestyle exposure on described placental marker of development and function; main result in green = positive impact of periconceptional lifestyle exposure on described placental marker of development and function.

Table 2. Summary of the associations between maternal periconceptional alcohol exposure and markers of placental development and function (5 studies)

Author	Study design	Sample size	Exposure definition	Placental parameter	Definition of marker of placental development and function	Main result(s)
Vuorela *et al.,* 2002[121]	Prospective cohort study	82	Amount of maternal alcohol use per occasion and frequency of occasions. Heavy drinking: ≥8 drinks/week	Serum biomarker	Second-trimester PlGF (ng/L)	>
					Third-trimester PlGF (ng/L)	>
Avagliano *et al.,* 2012[55]	Cross-sectional	203	Undefined	Histology	Abnormal spiral artery modification: intact vessel wall without evidence of trophoblast invasion in at least 70% of the arterial sections	=
Tikellis *et al.,* 2012[114]	Prospective cohort study	7,945	Maternal alcohol use during pregnancy: yes/no	Histology	Placental weight (grams; not described)	<
Wang *et al.,* 2014[123]	Prospective cohort study	7,945	Number of maternal alcoholic drinks/day: none, 0-1 drinks/day, 2-3 drinks/day, 4-5 drinks/day, >6 drinks/day	Histology	Placental weight (grams; weighed wet after trimming cord and without removing membranes and blood clots)	=
					Placenta-to-birth weight ratio	<
					Classification by visual inspection as normal, incomplete, infarcted, post mature, clots on maternal side	=
					Other placental abnormality	=
Carter *et al.,* 2016[62]	Prospective cohort study	103	Maternal alcohol use at time of conception, recruitment and throughout pregnancy (at least 2 consumptions in past 2 weeks)	Histology	Placental weight (grams)	<
					Umbilical cord (number of vessels and coiling)	=
					Placental infarction/hemorrhage	>
					Placental infection/inflammation	=
					Plate vessel congestion	=
					Villous maturation	>
					Maternal placental vascular underperfusion	=
					Chorangiosis	=

BMI= body-mass index (kg/m², unless stated otherwise); L= liter; ng= nanogram; PlGF= placental growth factor

Main result in red= negative impact of periconceptional lifestyle exposure on described placental marker of development and function; main result in orange = no impact of periconceptional lifestyle exposure on described placental marker of development and function; main result in green = positive impact of periconceptional lifestyle exposure on described placental marker of development and function.

Table 3. Summary of the associations between maternal periconceptional nutrition and markers of placental development and function (12 studies)

Author	Study design	Sample size	Exposure definition	Placental parameter	Definition of marker of placental development and function	Main result(s)
Owens *et al.,* 2015[103]	Randomized controlled trial	376	Maternal preconceptional use of micronutrient supplement (15 vitamins and trace elements, i.e., vitamins A (800 retinol equivalents), D (200 IU), E (10 mg), C (70 mg), thiamin (1.4 mg), riboflavin (1.4 mg), niacin (18 mg), pryroxidine (1.9 mg), cobalamin (2.6mg), folic acid (400 μg), iron (30 mg) zinc (15 mg), copper (2 mg) selenium (65 μg) and iodine (150 μg)	Ultrasound	Second- and third-trimester high resistance waveforms: mean UtA RI >0.55 and bilateral notching or UtA RI >0.65 and unilateral notching	<
Timmermans *et al.,* 2011[116]	Prospective cohort study	5,993	Maternal folic acid use (0.4-0.5 mg): preconceptional start or start before 8 weeks GA	Ultrasound	Second and third-trimester UtA PI	<
					Second and third-trimester UtA RI	<
					Second and third-trimester UmbA PI	<
Timmermans *et al.,* 2012[117]	Prospective cohort study	3,207	Maternal nutritional intake in the first trimester (adherence to Mediterranean diet)	Ultrasound	Second- and third-trimester UtA RI	<
					Second- and third-trimester UmbA PI	<
Bautista Niño *et al.,* 2015[58]	Prospective cohort study	3,134	Maternal first-trimester dietary intake (including fish consumption) assessed by a Food Frequency Questionnaire (FFQ)	Serum biomarker	PlGF (in pg/mL) (first and second trimester, at birth)	=
					sFlt-1 (in ng/mL) (first and second trimester, at birth)	=
					sFlt-1/PlGF ratio (first and second trimester, at birth)	=
Fowles *et al.,* 2012[72]	Cross-sectional	118	Maternal first-trimester intake assessed by 24h dietary using the Nutrition Data System for Research created by the Nutrition Coordinating Center at the University of Minnesota	Serum biomarker	First-trimester angiogenic to antiangiogenic ratio [PlGF/(sFlt 1*sEng)]	> (for micro nutrients)
						< (for transfats)
Abreu *et al.,* 2016[52]	Prospective cohort study	98	Maternal dietary intake: three-day food diary (including 2 weekdays plus one weekend day in each trimester)	Histology	Placental weight (grams)	> (dairy intake)
Gernand *et al.,* 2015[75]	Randomized controlled trial	396	Maternal first-trimester initiation of multiple micronutrient supplementation: 15-vitamin and mineral supplement	Histology	Placental weight (grams)	=
Langley-Evans *et al.,* 2003[88]	Prospective cohort study	204	Maternal first-trimester five-day food diary of all nutrients	Histology	Placental weight (grams)	=

Table 3, continued

Author	Study design	Sample size	Exposure definition	Placental parameter	Definition of marker of placental development and function	Main result(s)
Moore *et al.,* 2004[98]	Prospective cohort study	557	Maternal first- (and third-) trimester food frequency questionnaire	Histology	Placental weight (grams)	> (percentage of energy from protein in early pregnancy)
Timmermans *et al.,* 2009[115]	Prospective cohort study	6,353	Maternal folic acid use (0.4-0.5 mg): preconceptional start or start before 8 weeks GA	Histology	Placental weight (grams)	> (pre-conceptional start)
Timmermans *et al.,* 2012[117]	Prospective cohort study	3,207	Maternal nutritional intake in the first trimester	Histology	Placental weight (grams)	< (lower adherence to Mediterranean diet)
Roseboom *et al.,* 2011[108]	Case-control	1,046	Maternal first-trimester famine (daily caloric intake <1000 calories during thirteen weeks of gestation)	Histology	Placental length (cm)	< (boys: pre-conceptional, early gestation)
					Placental breadth (cm)	< (boys: pre-conceptional, early gestation girls: pre-conceptional)
					Placental thickness (cm)	< (boys: pre-conceptional, early gestation girls: pre-conceptional)
					Placental area (cm²)	< (boys: pre-conceptional, early gestation, girls: pre-conceptional)
					Placental volume (cm²)	< (boys: early gestation)

Cm= centimeter; cm²= squared centimeters; IU= international units; μg= microgram; mg= milligram; mL= milliliter; mm= millimeter; ng= nanogram; PI= pulsatility index; PlGF= placental growth factor; pg= picogram; sFlt= soluble fms-like tyrosine kinase; UmbA= Umbilical artery; UtA= Uterine artery

Main result in red= negative impact of periconceptional lifestyle exposure on described placental marker of development and function; main result in orange = no impact of periconceptional lifestyle exposure on described placental marker of development and function; main result in green = positive impact of periconceptional lifestyle exposure on described placental marker of development and function.

Table 4. Summary of the associations between periconceptional maternal body weight and markers of placental development and function (33 studies)

Author	Study design	Sample size	Exposure definition	Placental parameter	Definition of marker of placental development and function	Main result(s)
Kinare *et al.,* 2000[86]	Prospective cohort study	487	Monthly pre-pregnancy maternal weight (kg)	Ultrasound	Mid-pregnancy placental volume (mL)	> (higher maternal body weight)
Thame *et al.,* 2000[113]	Prospective cohort study	428	First-trimester maternal weight (kg)	Ultrasound	Second-trimester placental volume (cm³)	> (higher maternal body weight)
Wills et al., 2010[124]	Prospective cohort study	478	Maternal BMI measured every 3 months up to pregnancy	Ultrasound	Second- and third-trimester placental volume (ml)	> (higher maternal BMI)
Faupel-Badger *et al.,* 2011[71]	Prospective cohort study	182	First-trimester maternal BMI (≤24.3, 24.4-28.7, >28.8)	Serum biomarker	First- and second-trimester PlGF (in pg/ml)	=
					First- and second-trimester sFlt1 (in pg/ml)	> (higher maternal body weight)
					First- and second-trimester sEng (in ng/ml)	< (higher maternal body weight)
					First- and second-trimester sFlt1/PlGF anti-angiogenic ratio	> (higher maternal body weight)
Mijal *et al.,* 2011[97]	Prospective cohort study	668	Pre-pregnancy maternal BMI classified as underweight, normal, overweight and obese (according to Centers for Disease Control guidelines)	Serum biomarker	Second-trimester PlGF levels (ng/ml)	< (higher maternal body weight)
					Second-trimester sFlt1 levels (pg/ml)	< (higher maternal body weight)
					Second-trimester sEng levels (pg/ml)	< (higher maternal body weight)
Zera *et al.,* 2014[126]	Prospective cohort study	2,399	First trimester normal maternal weight (BMI <25), overweight (BMI 25-30), obesity (BMI ≥30)	Serum biomarker	PlGF levels (ng/ml) in second and third trimester	< (higher maternal body weight)
					sFlt1 levels (pg/ml) in all trimesters	< (higher maternal body weight)
					Change rate in PlGF in all trimesters	< (higher maternal body weight)
Diouf *et al.,* 2014[68]	Prospective cohort study	1,744	Pre-pregnancy thin (maternal BMI <18.5), normal (maternal BMI 18.5-25), overweight (maternal BMI 25-30) and obese (maternal BMI ≥30)	Histology	Placental weight (grams)	> (higher maternal body weight)
Ditchfield *et al.,* 2015[69]	Cross-sectional	61	First-trimester ideal maternal weight (BMI 18.5-24.9) and obese (BMI ≥30)	Histology	Placental weight (grams)	=
Dubé et al., 2012[70]	Prospective cohort study	150	Pre-pregnancy normal weight (BMI 18.5-24.9) and obese (BMI >30)	Histology	Placental weight (grams)	> (higher maternal body weight)

Table 4, continued

Author	Study design	Sample size	Exposure definition	Placental parameter	Definition of marker of placental development and function	Main result(s)
Gernand *et al.,* 2012[74]	Randomized controlled trial	350	First-trimester maternal BMI <18.5, 18.5-24.9, ≥25.0	Histology	Placental weight (grams)	> (higher maternal body weight)
L'Abée *et al.,* 2011[87]	Prospective cohort study	2,947	Parental pre-pregnancy maternal BMI in kg/m2	Histology	Placental weight (grams)	> (higher maternal body weight)
Lappas *et al.,* 2014[90]	Case-control	29	Pre-pregnancy lean (maternal BMI 18-25) or obese (maternal BMI >30)	Histology	Placental weight (grams)	> (higher maternal body weight)
Little *et al.,* 2003[93]	Cross-sectional	1,621	Pre-pregnancy maternal BMI <19, BMI 19-22, BMI 23-26, BMI 27-29, BMI 30+	Histology	Placental weight (grams)	> (higher maternal body weight)
Mamun *et al.,* 2011[30]	Prospective cohort study	6,528	Pre-pregnancy normal maternal BMI (<25), overweight (25-29) and obese (≥30) using the WHO classification of BMI cut-offs	Histology	Placental weight (grams)	< (maternal underweight) > (maternal overweight and obesity)
McNamara *et al.,* 2014[96]	Retrospective cohort study	97,600	Pre-pregnancy maternal BMI (<18.5; 18.5-24.9; 26-29.9; ≥30)	Histology	Placental weight (grams)	> (higher maternal body weight)
Strøm-Roum *et al.,*2016[112]	Retrospective cohort study	106,191	Pre-pregnancy lean (maternal BMI 19-24) and obese (maternal BMI >32-43)	Histology	Placental weight (grams)	> (higher maternal body weight)
Saben *et al.,* 2014[109]	Case-control	24	First-trimester maternal BMI (<18.5; ≥18.5-<52, ≥25-<30, ≥30-<35 and ≥35)	Histology	Placental weight (grams)	=
Tikellis *et al.,* 2012[114]	Prospective cohort study	7,945	Maternal underweight: BMI <20, healthy weight: BMI 20-24.99, overweight: BMI 25-29.99, obese: BMI >30	Histology	Placental weight (grams; not described)	> (higher maternal body weight)
Tsai *et al.,* 2015[118]	Case-control	84	Lean (maternal BMI 16.0-24.9), overweight (maternal BMI 25.0-29.9) and obese (maternal BMI >30)	Histology	Placental weight (grams)	> (higher maternal body weight)
Wallace *et al.,* 2014[122]	Retrospective cohort study	12,740	Inter pregnancy change in maternal BMI (loss greater than 1 unit; -1 to +1 as BMI stable; 1- <3 as modest gain; >3 as large gain)	Histology	Placental weight <10th centile (weight loss) Placental weight >90th centile (weight gain)	< (higher maternal body weight) > (higher maternal body weight)

Table 4, continued

Author	Study design	Sample size	Exposure definition	Placental parameter	Definition of marker of placental development and function	Main result(s)
Baptiste-Roberts *et al.,* 2008[56]	Prospective cohort study	23,420	Pre-pregnancy maternal BMI (not defined)	Histology	Placental weight (grams)	> (higher maternal body weight)
					Placental growth restriction (<10th centile)	< (higher maternal body weight)
					Placental thickness (mm)	< (higher maternal body weight)
					Placental chorionic plate area (cm^2)	< (higher maternal body weight)
					Placental weight (grams)	> (more maternal weight gain)
					Placental growth restriction (<10th centile)	< (more maternal weight gain)
					Placental thickness (mm)	< (more maternal weight gain)
					Placental chorionic plate area (cm^2)	< (more maternal weight gain)
Bar *et al.,* 2012[57]	Matched case-control	56	Pre-pregnancy normal maternal weight (undefined) and obese (BMI ≥30)	Histology	Placental weight (grams)	=
					Placental inflammatory lesions	> (higher maternal body weight)
					Fetal vascular supply lesions	=
					Maternal vascular supply lesions	=
Berglund *et al.,* 2016[31]	Prospective cohort study	331	Pre-pregnancy normal maternal weight (18.5≤ BMI <25), overweight (25≤ BMI <30) and obese (BMI ≥30)	Histology	Placental weight (grams)	> (higher maternal body weight)
					Placental/fetal ratio	> (higher maternal body weight)
He *et al.,* 2016[77]	Case-control	92	Pre-pregnancy lean (maternal BMI <25) or obese (maternal BMI >30)	Histology	Large-for-gestational age placenta	> (higher maternal body weight)
					Placental inflammation	> (higher maternal body weight)
					Marginal umbilical cord insertion	> (higher maternal body weight)
					Intervillous thrombi (placental parenchyma)	> (higher maternal body weight)
					Combined maternal and fetal inflammatory response	> (higher maternal body weight)

Author	Study design	Sample size	Exposure definition	Placental parameter	Definition of marker of placental development and function	Main result(s)
Huang *et al.,* 2014[79]	Prospective cohort study	39,774	Pre-pregnancy maternal underweight (BMI <18.5), normal weight (18.5-24.9), overweight (25.0-29.9) and obese (≥30) according to the WHO classification	Histology	Overweight: higher placenta weight-to-birthweight ratio	>
					Overweight: maternal vascular Infarct	>
					Overweight: >3 maternal infarcts	>
					Overweight: maternal thrombosis in cut surface	>
					Overweight: maternal vessels atheroma in decidua	>
					Overweight: maternal villous Infarcts	>
					Overweight: fetal neutrophilic infiltration	>
					Overweight: meconium of fetal membrane	>
					Underweight: small-for-gestational age placenta	>
					Underweight: fibrin deposition of the fetal membranes	>
					Underweight: placental maturity	>
Macdonald *et al.,* 2014[94]	Cross-sectional	2,016	Pre-pregnancy maternal BMI (<18.5; 18.5-24.9; 26-29.9; ≥30)	Histology	Underweight: PWR <10th centile in term pregnancies	>
					Overweight: PWR <10th centile in term pregnancies	>
					Overweight: PWR >90th centile in term pregnancies	<
					Obesity: PWR <10th centile in term pregnancies	>
					Obesity: PWR >90th centile in term pregnancies	<
Mando *et al.,* 2016[95]	Case-control	856	Pre-pregnancy normal maternal weight (18≤ BMI <25), overweight (25≤ BMI <30) and obese (BMI ≥30)	Histology	Placental weight (grams)	> (higher maternal body weight)
					Placental thickness	> (higher maternal body weight)
					Fetal-placental ratio	< (higher maternal body weight)

Table 4, continued

Author	Study design	Sample size	Exposure definition	Placental parameter	Definition of marker of placental development and function	Main result(s)
Wang *et al.*, 2014[123]	Prospective cohort study	7,945	Pre-pregnancy maternal BMI	Histology	Placental weight (grams; weighed wet after trimming cord and without removing membranes and blood clots)	> (higher maternal body weight)
					Placenta-to-birth weight ratio	=
					Classification by visual inspection as normal, incomplete, infarcted, post mature, clots on maternal side	=
					Other placental abnormality	=
Avagliano *et al.*, 2012[55]	Cross-sectional	203	Pre-pregnancy maternal obesity: BMI >30	Histology	Abnormal spiral artery modification: intact vessel wall without evidence of trophoblast invasion in at least 70% of the arterial sections	>
Desforges *et al.*, 2013[67]	Cross-sectional	?	First-trimester ideal maternal weight (BMI 18.5-24.9), overweight (BMI 25-29.9) or obesity (BMI >30)	Histology	Syncytiotrophoblast TauT activity	< (higher maternal body weight)
Higgins *et al.*, 2013[78]	Cross-sectional	24	First-trimester normal maternal weight (BMI 18.5-24.9), obesity class 1 and 2 (BMI 30.0-39.9) and obesity class 3 (BMI >40)	Histology	Placental villous proliferation	< (higher maternal body weight)
					Placental apoptosis	< (higher maternal body weight)
Loardi *et al.*, 2015[33]	Case-control	20	Pre-pregnancy lean (maternal BMI 19-25) or obese (maternal BMI 30-35)	Histology	Villous tree immaturity	> (higher maternal body weight)
					Placental inflammatory status	=
					Placental vascular pathology	=
Uhl *et al.*, 2015[119]	Case-control	48	Pre-pregnancy normal maternal weight (BMI 18.0-24.9) or obesity (BMI >30)	Histology	Placental phospholipids (i.e., placental transfer of fatty acids)	< (higher maternal body weight)

BMI= body-mass index (kg/m², unless stated otherwise); cm= centimeter; fβhCG= fetal beta human chorionic gonadotropin; IU/l= international units per liter; kg= kilogram; m= meter; μm= micrometer; mL= milliliter; mm= millimeter; MoM= multiples of the median; ng= nanogram; PAPP-A= pregnancy-associated plasma protein A; PI= pulsatility index; PlGF= placental growth factor; pg= picogram; PWR= placental weight ratio; RI= resistance index; sFlt= soluble fms-like tyrosine kinase; sEng= soluble endoglin; U/l= units per liter.

Main result in red= negative impact of periconceptional lifestyle exposure on described placental marker of development and function; main result in orange = no impact of periconceptional lifestyle exposure on described placental marker of development and function; main result in green = positive impact of periconceptional lifestyle exposure on described placental marker of development and function.

Table 5. Calculated odds ratios for markers of placental development and function in association with exposure to lifestyle

Periconceptional lifestyle Exposure	Placental marker of development and function	GA at outcome assessment	Number of participants	Odds ratio	95% CI
Maternal smoking	Higher UtA RI (Kho et al., 2009)[85]	20 weeks	2,459	1.21	0.78, 1.89
	Higher UmbA RI (Kho et al., 2009)[85]	20 weeks	2,459	1.62	1.03, 2.54*
	Placental-weight-ratio < p10 (Macdonald et al., 2014)[94]		20,216		
	• Preterm pregnancies	<33 weeks		0.94	0.49, 1.78
		≥33 - <37 weeks		0.49	0.26, 0.95*
	• Term pregnancies	≥37 weeks		0.65	0.56, 0.75*
	Placental-weight-ratio > p90 (Macdonald et al., 2014)[94]		20,216		
	• Preterm pregnancies	<33 weeks		0.63	0.27, 1.47
		≥33 - <37 weeks		1.52	0.97, 2.38
	• Term pregnancies	≥37 weeks		1.80	1.62, 2.00*
	Chorangiosis (Akbulut et al., 2009)[53]	At birth	61	7.26	0.84, 63.24
	Vasculosyncytial membrane thickening (Akbulut et al., 2009)[53]	At birth	61	60.00	12.23, 294.31*
Maternal second hand smoking	Lower placental weight (> 500 vs. < 500 grams) (Niu et al., 2016)[102]	At birth	390	2.30	1.10, 4.81*
Maternal alcohol use	Fetal thrombotic vasculopathy (Carter et al., 2016)	At birth	103	1.72	0.07, 43.23
Maternal obesity	Small-for-gestational age placenta (He et al., 2016)[77]	At birth	92	0.41	0.18, 0.96
	Fetal/placental weight ratio < p10 (He et al., 2016)[77]	At birth	92	1.79	0.49, 6.60
	Placental-weight-ratio < p10 (Macdonald et al., 2014)[94]		20,216		
	• Preterm pregnancies	<33 weeks		0.31	0.10, 0.94*
		≥33 - <37 weeks		0.71	0.26, 1.90
	• Term pregnancies	≥37 weeks		0.81	0.67, 0.97*
	Fetal/placental weight ratio > p90 (He et al., 2016)[77]	At birth	92	0.95	0.34, 2.65
	Placental-weight-ratio > p90 (Macdonald et al., 2014)[94]		20,216		

Table 5, continued

Periconceptional lifestyle Exposure	Placental marker of development and function	GA at outcome assessment	Number of participants	Odds ratio	95% CI
Maternal obesity *(continued)*	• Preterm pregnancies	<33 weeks		0.81	0.28, 2.40
		≥33 - <37 Weeks		2.33	1.14, 4.74*
	• Term pregnancies	≥37 weeks		1.52	1.30, 1.79*
	Large-for-gestational age placentas (He *et al.,* 2016)[77]	At birth	92	7.37	1.55, 35.15*
	Higher placenta-to-birth weight ratio (Huang *et al.,* 2014)[79]	At birth	39,774	1.25	1.09, 1.42*
	Villous tree immaturity (Loardi *et al.,* 2015)[33]	At birth	20	71.40	3.00, 1696.84*
	Maternal villous infarcts (Huang *et al.,* 2014)[79]	At birth	39,774	1.18	1.04, 1.35*
	Intervillous thrombi parenchyma (He *et al.,* 2016)[77]	At birth	92	8.22	1.74, 38.93*
	Vascular lesions of maternal origin (Huang *et al.,* 2014)[79]	At birth	39,774		
	• Vascular infarct			1.37	1.18, 1.60*
	• > 3 Infarcts			1.28	1.03, 1.58*
	• Thrombosis in cut surface			1.52	1.34, 1.73*
	• Vessels atheroma in decidua			1.97	1.11, 3.50*
	Maternal vascular pathology (Bar *et al.,* 2012)[57]	At birth	56	0.75	0.26, 2.15
	Maternal inflammatory response (Bar *et al.,* 2012; He *et al.,* 2016)[57,77]	At birth	148	1.79	0.82, 3.93†
	Combined maternal and fetal inflammatory response (He *et al.,* 2016)[77]	At birth	92	3.92	1.17, 13.13*
	Fetal neutrophilic infiltration (Huang *et al.,* 2014)[79]	At birth	39,774	1.26	1.08, 1.48*
	Meconium of fetal membrane (Huang *et al.,* 2014)[79]	At birth	39,774	1.60	1.39, 1.84*
	Abnormal cord coiling (He *et al.,* 2016)[77]	At birth	92	1.96	0.17, 22.35
	Single umbilical cord artery (He *et al.,* 2016)[77]	At birth	92	0.96	0.06, 15.77
	Marginal umbilical cord insertion (He *et al.,* 2016)[77]	At birth	92	5.81	1.20, 28.23*

Table 5, continued

Periconceptional lifestyle Exposure	Placental marker of development and function	GA at outcome assessment	Number of participants	Odds ratio	95% CI
Maternal overweight *(continued)*	Placental-weight-ratio < p10 (Macdonald *et al.,* 2014)[94]		20,216		
	• Preterm pregnancies	<33 weeks		0.42	0.15, 1.14
		≥33 - <37 weeks		0.80	0.38, 1.72
	• Term pregnancies	≥37 weeks		0.82	0.71, 0.97*
	Placental-weight-ratio > p90 (Macdonald *et al.,* 2014)[94]		20,216		
	• Preterm pregnancies	<33 weeks		0.86	0.29, 2.56
		≥33 - <37 weeks		1.14	0.55, 2.36
	• Term pregnancies	≥37 weeks		1.22	1.05, 1.41*
Maternal higher BMI	Abnormal spiral artery remodeling (Avagliano *et al.,* 2012)[55]	At birth	203	1.10	1.01, 1.20*
Maternal underweight	Small-for-gestational age placenta (Huang *et al.,* 2014)[79]	At birth	39,774	1.74	1.57, 1.92*
	Placental-weight-ratio < p10 (Macdonald *et al.,* 2014)[94]		20,216		
	• Preterm pregnancies	<33 weeks		0.57	0.31, 1.03
		≥33 - <37 weeks		1.02	0.61, 1.70
	• Term pregnancies	≥37 weeks		0.92	0.83, 1.03
	Placental-weight-ratio > p90 (Macdonald *et al.,* 2014)[94]		20,216		
	• Preterm pregnancies	<33 weeks		0.90	0.43, 1.89
		≥33 - <37 weeks		1.49	0.89, 2.51
	• Term pregnancies	≥37 weeks		1.30	1.16, 1.45*
	Placental immaturity (Huang *et al.,* 2014)[79]	At birth	39,774	1.28	1.14, 1.44*
	Fibrin deposition of fetal membranes (Huang *et al.,* 2014)[79]	At birth	39,774	1.15	1.01, 1.31*
< -1 points inter pregnancy maternal BMI loss	Placental weight < p10 (Wallace *et al.,* 2014)[122]	At birth	12,740	1.67	1.35, 2.07*
	Placental weight > p90 (Wallace *et al.,* 2014)[122]			0.90	0.72, 1.11
-1 to <1 point inter pregnancy maternal BMI change	Placental weight < p10 (Wallace *et al.,* 2014)[122]	At birth	12,740	0.91	0.76, 1.09
	Placental weight > p90 (Wallace *et al.,* 2014)[122]			1.26	1.08, 1.47*
>3 points inter pregnancy maternal BMI gain	Placental weight < p10 (Wallace *et al.,* 2014)[122]	At birth	12,740	0.99	0.77, 1.28
	Placental weight > p90 (Wallace *et al.,* 2014)[122]			1.59	1.31, 1.47*

Significant at p<0.05; † pooled odds ratio

BMI = body-mass index in kg/m²; CI = confidence interval; GA = gestational age; p10 = 10th centile; p90 = 90th centile; PI = pulsatility index; RI = resistance index; UmbA = Umbilical Artery; UtA = Uterine Artery

DISCUSSION

Main findings and implications
In this systematic review, we provide evidence of the influence of periconceptional maternal smoking, alcohol use, nutrition and maternal body weight on several clinical features and biomarkers of placental development and function throughout pregnancy. However, placental functionality both depends on development and adaptive capacity[128]. Therefore, developmental changes in the placenta are not necessarily correlated with alterations in placental function.

Most evidence was available on the negative impact of maternal periconceptional smoking on placental ultrasound markers and serum biomarkers, and less on placental histology[63,64,80-82,85,97,125]. The negative associations could not always be extrapolated to third-trimester placental weight or other placental histological features[87,101,102,123]. Jauniaux et al., however, showed associations in a previous review between smoking during pregnancy and a decreased mean placental weight and an increase of gross morphological changes, such as infarctions and microstructural changes of the placental villi[11]. One explanation for these contradictory findings is that following smoking cessation catch-up growth of the placenta during pregnancy, comparable to the catch-up growth observed in FGR after pregnancy, can occur. In addition, smoking cessation later in pregnancy and/or passive smoking in early pregnancy may have distorted the effect estimates of the other studied placental markers.

Additional evidence for the impact of lifestyle factors on placental development and function is available from animal studies, illustrated by the following studies. De Souza *et al.* reported that cigarette smoke exposure in female rats led to lower fetal weight, but higher placental weight, indicative of a placental compensatory mechanism[129]. Another study in baboons revealed abnormal growth and development of the fetoplacental arterial tree, resulting from preconceptional polycyclic aromatic hydrocarbon exposure at levels typically caused by cigarette smoking[130]. Periconceptional exposure to a binge-drinking level of alcohol in mice resulted in placental anomalies, i.e. altered Doppler waveform of the umbilical artery and lower placental weights at birth[131]. Moreover, periconceptional undernutrition in singleton pregnancies of ewes increased placental weight, also indicating a compensatory mechanism to limited nutrient availability[132]. Caffeine exposure during pregnancy in rats revealed that gene expression of B-cell CLL/lymphoma 2 (Bcl-2), an anti-apoptosis regulator, was decreased in the placenta. This is an interesting finding because decreased Bcl-2 expression has been reported in placentas of IUGR pregnancies[133].

Epigenetics was not within the scope of our review. However, of interest to mention is that more data are becoming available on associations between maternal smoking during pregnancy and variations in DNA methylation profiles of not only the neonatal epigenome, but also of the placental genome[134,135]. This is an important finding and supports the detrimental long-term health and transgenerational health effects of lifestyle exposure[2].

Although definitions in timing and the amount of maternal exposure to alcohol vary throughout the included studies, associations are shown between maternal periconceptional alcohol use and a decreased placental weight as marker of poor development and function[62,114]. The association

observed between maternal alcohol abuse in the second and third trimesters of pregnancy and higher levels of PlGF can be explained as ethanol-induced alterations in placental PlGF expression or release[121]. No clear changes in placental histology at birth were found[55,62,123]. In addition, in vitro research demonstrated associations between ethanol-induced programmed trophoblast cell death and altered placental transport of nutrients[62]. This data suggest that periconceptional maternal alcohol exposure can affect placental development and function, but the time frame of exposure and amount of alcohol use are critical to assess its actual impact.

This review provides evidence about the impact of maternal nutrient intake on placental health. Periconceptional maternal synthetic folic acid supplement intake and strong adherence to a natural folate-rich Mediterranean diet were associated with an increased placental weight[52,98,116,117]. In contrast, a low caloric intake resulted in smaller placental measures[108]. A previous literature review demonstrated that a maternal dietary pattern adequate in either energy, protein, essential fatty acid or folate levels before conception and during pregnancy significantly diminished adverse birth outcomes, such as FGR and PTB[136]. Even in the early stages of pregnancy, associations were found between increased embryonic growth and energy-rich dietary patterns, dietary patterns high in fish and olive oil, and also folic acid supplement intake[137-139]. Since nutrition like other lifestyle factors is modifiable, it is clear that more research is needed to assess the nutritional needs for placental development and function in order to prevent adverse placenta-related outcomes in mother and offspring of general and high-risk populations[140].

Obesity not only results from an unhealthy dietary pattern, typically characterized by a high-caloric intake and low intake of vitamin, but also from a sedentary lifestyle. Maternal BMI as phenotype can therefore be considered a proxy of the health status. In general, a higher maternal BMI is associated with a larger placental volume assessed by ultrasound[86,113,124] and a larger placental weight at birth which is a proxy for long-term health of the offspring[30,56,68,77,86,87,93,96,112-114,122-124]. In addition to the associations with placental size measurements, a negative association between maternal BMI and functional parameters was also demonstrated, i.e., decreased PlGF-serum levels[97,126]. These findings imply that modification of maternal BMI can alter placental development. This is an important finding with regard to the burden of maternal obesity during pregnancy and emphasizes the need for effective lifestyle or social interventions[141].

Lifestyle factors are different within and between individuals, families, populations and countries and are very much influenced by other conditions, such as age, reproductive behavior, psychological and societal factors like education, work and housing, for which we adjusted in this review[142-145]. Harmful societal factors accumulate in vulnerable women and migrants showing increased rates of maternal and perinatal mortality and morbidity, which are also due to placental-related complications[146,147]. Unfortunately, only a few studies provided information about societal factors that also strongly inform which lifestyle interventions are feasible and what strategies should be used for intervention. Therefore, we recommend that future studies should address societal factors in more detail.

As mentioned, we are very much aware that besides the studied lifestyle factors other maternal- and environmental-related factors could have further affected the effect estimates found in this review. However, these maternal and environmental factors are less likely to augment chances of

placenta-related pregnancy complications, mediated by an increase in prevalence of cardiovascular risk factors and they are less prone to modification on an individual level. Consequently, we left maternal and environmental factors out of the scope of this review. However, we assessed whether adjustments in analyses were made for these factors as covariates.

The strengths of this systematic review are the extensive literature search and the check of cross-references to minimize the odds of missing any eligible studies. Because we have chosen to provide a detailed overview of markers with known clinical applicability the excluded studies are those investigating placental markers in a research setting, such as epigenetic makers. Our study revealed that data on actual *in vivo* assessment of placental development and function in ongoing pregnancy is limited. Therefore, most available placental markers served as indirect and retrospective measures, such as placental histological characteristics and placental weight at birth. Although it was our intention to perform a meta-analysis, this was not feasible due to the large heterogeneity in lifestyles and placental markers studied.

In the interpretation of the data we have to address the following methodological issues. For inclusion, the time window of lifestyle exposure in studies had to cover at least a part of the periconception period. In many studies, the period of exposure continued later into gestation and this could have affected the estimates of the associations. The reliability of methods used to assess lifestyle factors varied strongly. Many studies used non-validated food frequency questionnaires or questioned periconceptional lifestyle retrospectively. In addition, the use of self-administered questionnaires introduced recall bias and socially desirable answers, which is in contrast to the use of medical records and serum/urine measurements, e.g., cotinine levels to assess smoking status. Also residual confounding could not be excluded in several studies, since mainly observational cohort studies or case-control studies with a cross-sectional design were retrieved. Moreover, in many studies the sample size was small and therefore underpowered for sophisticated statistical analysis.

We are aware that in this review some studies are included using the same cohorts of patients to investigate associations between various maternal lifestyle and/or clinical features and biomarkers of placental function and development. However, most studies addressed different lifestyles and placental markers. In four studies the cohort was extended or different subgroups of the cohort were studied[63,64,101,102]. In the absence of a meta-analysis, we chose to present the results of all these four studies, which carries the risk of overestimation of the associations found. Overall, the studies included in this review demonstrate that lifestyle factors and markers of placental development and function were not always clearly defined, and definitions differed often significantly between studies. Therefore, cautious interpretation of the directions of the associations and estimates is needed.

Future research
We identified several knowledge gaps regarding the evidence of the impact of periconceptional maternal lifestyle on placental development and function. Here we summarize some methodological issues and topics for further investigation. Longitudinal studies are scarce and should be conducted to investigate placental development and function by serial measurements

throughout pregnancy in order to minimize time gaps between the assessment of lifestyle exposures and placental outcomes. In addition, data comparison would be much easier and valid if definitions are harmonized by using the BMI categories defined by the World Health Organization (WHO, 1995) and performance of placental histological examination following the recently issued international guidelines[148]. Placental markers, such as placental weight, only partially reflect the actual impact of periconceptional maternal lifestyle on placental development and function throughout pregnancy. To adequately use placental markers as predictors for pregnancy outcome, we propose to develop personalized models, including patient characteristics and lifestyle exposures, validated in different general and high-risk populations[35,46-50,149]. This review shows that the knowledge to develop personalized models is lacking due to inconsistently measured data and we therefore propose consistent data collection and collaborations between group where possible.

A very interesting but so far largely neglected topic is the role of the preconceptional endometrium for optimal placentation. Endometrial receptivity precedes embryonic implantation and enables the physiological vascular transformation of the spiral arteries into low resistance vessels[9]. Failure of this transformation process is believed to be one of the causes of placenta-related complications[1,8,9]. Since an optimal endometrium is crucial for placentation, future research should address the impact of maternal lifestyle on the condition of the preconceptional endometrium. Although nowadays it is more feasible to recruit women preconceptionally, in particular subfertile women, it remains challenging to recruit fertile women[150,151].

One of the conclusions of this review is that interventions and strategies have to be developed to benefit placentation. Programs for folic acid supplement use, smoking cessation and optimizing dietary patterns are already available and can be used, in particular personal mHealth coaching programs are very much appreciated[140,152,153].

Ideally, all women with an intention to become pregnant, but moreover those women living in deprived neighbourhoods, should be offered a face-to-face preconceptional consultation via health care centers and e-consultation, to assess risk factors in lifestyle and nutrition and provide them with affordable tools to improve their lifestyle and nutritional behaviour[145,154-156]. Women living in deprived neighbourhoods may be less motivated and/or enabled to alter unhealthy lifestyle behaviours due to health illiteracy, lower education and limited finances. More easily accessible tools are mHealth applications to improve lifestyle and nutrition, since mobile phones have become broadly available[152]. Therefore, future research should aim to develop evidence-based, tailored interventions and strategies for these groups by incorporating the impact of societal factors in these tools.

In this review we did not search for paternal lifestyle, however, we encountered one study reporting an association between paternal weight and placental weight[87]. Because of the influence of paternal lifestyle on maternal lifestyle and the paternal (epi)genetic contribution to placental development and function, it would be valuable to address also paternal lifestyle in future studies.

Conclusions

This review summarizes the evidence of the impact of periconceptional maternal lifestyle on placental function and development. Because of the poorly defined exposures and time windows of investigation, unstandardized measurements of the outcome and small sample sizes of the included studies, a cautious interpretation of the effect estimates is needed. We identified several gaps of knowledge and emphasize that future research should focus more on the physiological consequences of (un)healthy maternal lifestyle on placentation during the critical periconception window. Moreover, we foresee that this new evidence will support the development of effective lifestyle interventions to further improve the health of mothers and their offspring from the earliest moment in life.

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ADDENDUM

Supplemental Table 1. Main characteristics of 82 included studies

Study	Year	Country	Study population	Study design	Sample size	Maternal exposure(s)	Outcome(s)	Quality score*
Abreu	2016	Portugal	Pregnant women attending the Sao Joao hospital in Porto, Portugal, July 2010 - May 2012	Prospective cohort study	98	Maternal nutrition	Histology	2
Akbulut	2009	Turkey	Uncomplicated, normal term, pregnant women delivering at the Denizli state hospital who had their second and third trimesters in winter months, March - May 2006	Cross-sectional	92	Maternal smoking	Histology	3
Altmäe	2016	Germany, Spain, Sweden	Women participating in the PREOBE study (role of nutrition and maternal genetics on the programming of development of fetal adipose tissue), conducted at the clinical university hospital San Cecilio and Mother-Infant hospital in Granada, Spain	Prospective cohort study	10	Maternal body weight	Biomarker	4
Avagliano	2012	Italy	Database of consecutive unselected perinatal autopsies at the San Paolo hospital medical school, cases of intra uterine fetal death ≥ 20 weeks GA, 2002 – 2011	Cross-sectional	203	Maternal alcohol use, Smoking, Body weight	Histology	3
Baptiste-Roberts	2008	USA	Black or Caucasian women who gave birth between 36 - 42 weeks GA included in the collaborative perinatal project (conducted in 12 hospitals across the USA), 1959 – 1966	Prospective cohort study	23,420	Maternal body weight	Histology	8
Bar	2012	Israel	An Edith Wolfson medical center database of placental analyses in complicated pregnancies (preeclampsia, fetal growth restriction or gestational diabetes) or randomly in uncomplicated pregnancies, January 2010 - July 2011	Matched case-control	56	Maternal body weight	Histology	3
Bautista Niño	2015	The Netherlands	Embedded in the Generation R study, a population-based cohort in Rotterdam, The Netherlands, including women in early pregnancy who were resident in the study area, delivering between April 2002 - January 2006	Prospective cohort study	3,134	Maternal nutrition	Biomarker	7
Berglund	2016	Spain	Pregnant women attending the clinical university San Cecilio and Mother-Infant university hospital of Granada, Spain, 2008 – 2012	Prospective cohort study	331	Maternal body weight	Histology	4
Bruchova	2010	Czech Republic	Women who gave birth to a full-term baby at the children hospital of Motol in Prague, June 2007 - October 2008	Cross-sectional	76	Maternal smoking	Histology	4

Study	Year	Country	Study population	Study design	Sample size	Maternal exposure(s)	Outcome(s)	Quality score*
Bush	2000	United Kingdom	Healthy Caucasian women with uncomplicated pregnancies from Aberdeen, recruited at approximately 13 weeks GA	Prospective cohort study	92	Maternal smoking	Histology	5
Bush	2000	United Kingdom	Healthy Caucasian women with uncomplicated pregnancies from Aberdeen, recruited at approximately 13 weeks GA	Prospective cohort study	?	Maternal smoking	Histology	5
Carter	2016	South Africa/USA	Pregnant women recruited from 3 antenatal midwife obstetric units that serve economically disadvantaged communities in Cape Town, South Africa, October 2011 - October 2014	Prospective cohort study	103	Maternal alcohol use, Smoking	Histology	6
Challier	2008	France	Women undergoing elective caesarean delivery at term (38 - 40 weeks GA) in the MetroHealth medical center	Cross-sectional	35	Maternal body weight	Histology, biomarker	3
Chelchowska	2012	Poland	Women with an uncomplicated, first-trimester pregnancy attending the medical university and institute of mother and child in Warsaw, Poland	Prospective cohort study	60	Maternal smoking	Biomarker	5
Chełchowska	2016	Poland	Women with an uncomplicated, first-trimester pregnancy attending the medical university and institute of mother and child in Warsaw, Poland, January 2010 - March 2012	Nested case-control	150	Maternal smoking	Biomarker	6
Chellakooty	2002	Denmark	All women with routine ultrasound examination in an outpatient clinic, GA determined by biparietal diameter between 14 - 22 weeks, November 1996 - June 1998	Prospective cohort study	455	Maternal smoking, Body weight	Biomarker	5
de B Machado	2011	Brazil	Pregnant women attending the hospital Sao Lucas da pontificia universidade catolica do Rio Grande do Sul, Porto Alegre, Brazil, September 2008 - September 2009	Prospective cohort study	64	Maternal smoking	Ultrasound	4
Desforges	2013	UK	First-trimester placentas (7 - 13 weeks GA) obtained following elective medical or surgical termination of pregnancy	Cross-sectional	?	Maternal body weight	Histology	2
Diouf	2014	France	Mother-child pairs enrolled in EDEN mother-child cohort (studying pre- and early post-natal determinants of child development and health), conducted at university hospitals in Nancy and Poitiers, France	Prospective cohort study	1,744	Maternal body weight	Histology	5

Supplemental table 1, continued

Study	Year	Country	Study population	Study design	Sample size	Maternal exposure(s)	Outcome(s)	Quality score*
Ditchfield	2015	United Kingdom	Placentas delivery in the St. Mary's hospital, Manchester, from full-term (37 - 42 weeks GA) uncomplicated pregnancies (between the 10th and 90th individualized birth weight centile)	Cross-sectional	61	Maternal body weight	Histology	3
Dubé	2012	Canada	Caucasian and healthy women recruited before their 10th week of pregnancy attending the clinique fidès or centre hospitalier de l'université de Montréal, Montréal, Canada, 2002 - 2006	Prospective cohort study	150	Maternal body weight	Histology	5
Faupel-Badger	2011	USA, Norway	Subset of participants with a second trimester fasting blood sample collection in the Massachusetts general hospital obstetric maternal study between 1998 – 2005	Prospective cohort study	182	Maternal body weight	Biomarker	5
Fowles	2012	USA	Sample of low-income women recruited from five clinics in central Texas, providing low-cost pregnancy testing	Cross-sectional	118	Maternal nutrition	Biomarker	5
Geelhoed	2011	Netherlands	Embedded in the Generation R study, a population-based cohort in Rotterdam, The Netherlands, including women in early pregnancy who were resident in the study area, delivering between April 2002 - January 2006	Prospective cohort study	1,120	Maternal smoking	Ultrasound	6
Genbacev	2000	USA	Group of women with early pregnancy loss between 6 - 12 weeks GA	Case- control	96	Maternal smoking	Histology, biomarker	3
Gernand	2012	Bangladesh, USA	Women were enrolled from an ongoing, community-based, cluster-randomized, controlled trial of maternal supplementation and received daily either an iron and folic acid (standard of care) or a 15-vitamin and mineral supplement from early pregnancy to 12 weeks postpartum	Randomized controlled trial	350	Maternal body weight	Histology	6
Gernand	2015	Bangladesh, USA	Women were enrolled from an ongoing, community-based, cluster-randomized, controlled trial of maternal supplementation and received daily either an iron and folic acid (standard of care) or a 15-vitamin and mineral supplement from early pregnancy to 12 weeks postpartum	Randomized controlled trial	396	Maternal nutrition	Histology, biomarker	10

Study	Year	Country	Study population	Study design	Sample size	Maternal exposure(s)	Outcome(s)	Quality score*
Gruslin	2001	Canada	Placentas were obtained after first or second trimester therapeutic terminations or after delivery in the third trimester	Cross-sectional	129	Maternal smoking	Histology	4
He	2016	USA	English-speaking and largely Caucasian women recruited at delivery, from an existing cohort conducted in a tertiary care obstetric hospital	Case-control	92	Maternal body weight	Histology	4
Higgins	2013	UK	Randomly selected, healthy women with known first-trimester BMI delivering between 37 - 42 weeks GA	Cross-sectional	24	Maternal body weight	Histology	2
Huang	2014	China, USA	Sub-cohort of women who gave birth between 36 - 42 weeks GA included in the collaborative perinatal project (conducted in 12 hospitals across the USA), 1959 – 1966	Prospective cohort study	39,774	Maternal body weight	Histology	8
Jauniaux	2013	Ireland, UK	Pregnant women booking for antenatal care at the university college London hospital, recruitment over 40 months	Prospective cohort study	128	Maternal smoking	Biomarker, ultrasound	4
Jeyabalan	2010	USA	Secondary analysis of the maternal-fetal medicine units network multicenter randomized controlled trial (conducted in 13 centers) of low-dose aspirin for the prevention of preeclampsia in high-risk women	Prospective cohort study	993	Maternal smoking	Biomarker	6
Kagan	2007	UK	Women screening for Down syndrome between 11 - 13+6 weeks GA at the Harold Wood hospital Essex and the fetal medicine centre, London	Retrospective cohort study	109,263	Maternal smoking	Biomarker	4
Kawashima	2014	Japan	Pregnant females requesting artificial abortion at 6 - 8 weeks GA at the Okayama clinic, Tokyo, Japan, and Kitamura clinic, Kawasaki, Japan, February - July 2014	Case-Control	52	Maternal smoking	Biomarker	4
Kawashima	2015	Japan	Pregnant females requesting artificial abortion before 7 - 8 weeks GA at the Okayama clinic, Tokyo, Japan, and Kitamura clinic, Kawasaki, Japan, February - July 2014	Case-Control	19	Maternal smoking	Biomarker	4
Kho	2009	Australia	Healthy nulliparous women with singleton pregnancies, participating in the SCOPE study in New Zealand/Australia, November 2004 - July 2007	Prospective cohort study	2,459	Maternal smoking	Ultrasound	7

Supplemental table 1, continued

Study	Year	Country	Study population	Study design	Sample size	Maternal exposure(s)	Outcome(s)	Quality score*
Kinare	2000	India, UK	Subgroup of pregnant women in a prospective community-based study on maternal nutrition and fetal growth in 6 Indian villages	Prospective cohort study	487	Maternal body weight	Histology, ultrasound	6
L'Abée	2011	The Netherlands	All children born between April 2006 - 2007, of pregnant women recruited in the third trimester, living in the province of Drenthe, The Netherlands at the time of birth	Prospective cohort study	2,947	Maternal smoking, Body weight	Histology	6
Langley-Evans	2003	United Kingdom	Randomly selected healthy white women attending the Northampton general hospital for an ultrasound dating in the first 15 weeks of pregnancy	Prospective cohort study	204	Maternal nutrition	Histology	5
Lappas	2014	Australia	Pregnant women at time of term caesarean section in the absence of labour	Case-Control	29	Maternal body weight	Histology, biomarker	3
Lappas	2014	Australia	Pregnant women at time of term caesarean section in the absence of labour, November 2011 - July 2013	Case-Control	60	Maternal body weight	Biomarker	4
Lassance	2015	USA	Pregnant women recruited at scheduled term caesarean section	Cross-sectional	234	Maternal body weight	Histology	5
Lesseur	2014	Canada	Mother-infant dyads with placental leptin methylation information, enrolled in the RICH study, only singleton, viable infants without congenital abnormalities, between September 2009 - October 2012	Prospective cohort study	535	Maternal smoking, Body weight	Histology	4
Little	2003	Ukraine	Women participating in children of Ukraine study, fluent in Ukrainian, divided in 6 geographic cohorts who delivered singleton infants of at least 20 weeks GA	Cross-sectional	1,621	Maternal body weight	Histology	5
Loardi	2015	Italy	Pregnant women undergoing pre-labour elective caesarean section under spinal anesthesia in the spedali civili, university of Brescia, Italy, March - July 2012	Case-Control	20	Maternal body weight	Histology	4
Macdonald	2014	Canada	Hospital-based births of 22 - 42 weeks GA in St. Joseph's health care and Victoria hospital, June 2006 - March 2011	Cross-sectional	20,216	Maternal smoking, Body weight	Histology	4

Study	Year	Country	Study population	Study design	Sample size	Maternal exposure(s)	Outcome(s)	Quality score*
Malti	2014	Algeria	Uncomplicated pregnant women, included at delivery by caesarean section in the Tlemcen hospital, Tlemcen, Algeria	Case-Control	90	Maternal body weight	Biomarker	3
Mamun	2011	Australia	Pregnancies as part of the master-university study of pregnancy birth cohort who received antenatal care at a major public hospital in Brisbane, Australia, 1981 – 1983	Prospective cohort study	6,528	Maternal body weight	Histology	8
Mando	2016	Italy	Uncomplicated pregnancies delivering at term (≥37 weeks GA) by vaginal delivery or caesarian section in the Sacco hospital, Milan, Italy, April 2010 - December 2013	Case-Control	856	Maternal body weight	Histology	6
McNamara	2014	Canada	Births without congenital anomalies between 24 - 43 weeks GA at the Royal Victoria hospital, Montréal, Canada, 1978 - 2007	Retrospective cohort study	87,600	Maternal smoking, Body weight	Histology	5
Mijal	2011	USA	Pregnant women that underwent maternal serum screening for α-fetoprotein, with singleton pregnancies and no known abnormalities, recruited from 52 prenatal clinics in 5 different Michigan communities between September 2008 - June 2004	Prospective cohort study	668	Maternal smoking, Body Weight	Biomarker	7
Moore	2004	Australia	Pregnant Caucasian women living in Adelaide, in the first 16 weeks of a singleton pregnancy	Prospective cohort study	557	Maternal nutrition	Histology	7
Morales	2016	Spain	Pregnant women participating in four cohorts in Asturias, Gipuzkoa, Sabadell and Valencia of the infancia y medio ambiente project in Spain, 2003 – 2008	Prospective cohort study	179	Maternal smoking	Histology, biomarker	6
Muralimanoharan	2015	USA	Uncomplicated, pregnant women at delivery by caesarean section in the university hospital San Antonio, Texas, USA	Case-Control	36	Maternal body weight	Histology, biomarker	3
Niu	2015	China, USA	Pregnant women without obstetric complications or congenital malformations attending the Foshan and Shenzhen women and children's hospitals in Guangdong, China, September 2009 - March 2011	Case-Control	2,143	Maternal smoking	Histology	7

Supplemental table 1, continued

Study	Year	Country	Study population	Study design	Sample size	Maternal exposure(s)	Outcome(s)	Quality score*
Niu	2016	China	Pregnant women without obstetric complications or congenital malformations attending the Foshan and Shenzhen women and children's hospitals in Guangdong, China, September 2009 - March 2010	Case-Control	390	Maternal smoking	Histology	6
Owens	2015	The Gambia, UK	Pre-pregnant women in 33 villages in Kiang West region of The Gambia, March 2006 - June 2008	Randomized controlled trial	376	Maternal nutrition	Ultrasound	8
Perdu	2016	Canada	Pregnant women undergoing elective termination of pregnancy between 5 - 13 weeks GA at the British Columbia's women's hospital, Vancouver, Canada	Prospective cohort study	90	Maternal body weight	Histology, biomarker	3
Prince	2017	USA	Uncomplicated, pregnant women at delivery by caesarean section in the university hospital San Antonio, Texas, USA	Case-Control	52	Maternal body weight	Histology, biomarker	3
Pringle	2005	Canada, Ireland, UK	Consecutive Caucasian pregnant women with their first prenatal visit before 20 weeks GA at university college London hospitals, April 1996 - July 1997	Case-Control	1,484	Maternal smoking	Histology, biomarker, ultrasound	5
Rizzo	2009	Italy	Pregnancies without congenital malformations and with successful recordings of placental volume and 3D vascularization, included as part of a project on placental development, università di Roma	Prospective cohort study	80	Maternal smoking, Body weight	Ultrasound	3
Roseboom	2011	The Netherlands, Saudi Arabia, UK	Infants in utero during famine in early gestation, who were born as singletons at term, 37 weeks GA or later, in the Wilhelmina gasthuis in Amsterdam, November 1943 - February 1947	Case-Control	1,046	Maternal nutrition	Histology	4
Saben	2014	USA	Placentas collected at birth at the university of Arkansas for medical sciences	Case-Control	24	Maternal body weight	Histology, biomarker	3
Shinjo	2014	Japan, Peru	Women without chronic disease, requesting surgical termination of pregnancy (at 6 - 7 and 10 - 11 weeks GA) at Showa university school of medicine	Case-Control	31	Maternal smoking	Biomarker	3
Stewart	2007	Scotland, UK	Lean and obese pregnant women registered for obstetric care at the Princess Royal maternity hospital (Glasgow) were recruited at their first visit at 10 - 12 weeks GA	Case-Control	60	Maternal body weight	Biomarker	4
Strøm-Roum	2016	Norway	Data from medical birth registry in Norway, 2009 – 2012	Retrospective cohort study	106,191	Maternal body weight	Histology	6

Study	Year	Country	Study population	Study design	Sample size	Maternal exposure(s)	Outcome(s)	Quality score*
Thame	2000	Jamaica, UK	Women at their first antenatal visit at the university hospitals of the West Indies, Kingston, Jamaica, between 15 - 40 years, with certain dates of their last menstrual period (confirmed by 14-week ultrasound), without systemic illnesses or genetic abnormalities	Prospective cohort study	428	Maternal body weight	Ultrasound	6
Tikellis	2012	Australia, UK	Embedded in the Tasmanian Infant Health Survey, a cohort investigating the cause of sudden infant death syndrome in Tasmania, Australia, January 1988 - December 1995	Prospective cohort study	7,945	Maternal alcohol use, Smoking, Body weight	Histology	5
Timmermans	2009	The Netherlands	Embedded in the Generation R study, a population-based cohort in Rotterdam, The Netherlands, including women in early pregnancy who were resident in the study area, delivering between April 2002 - January 2006	Prospective cohort study	6,353	Maternal nutrition	Histology	7
Timmermans	2011	The Netherlands	Embedded in the Generation R study, a population-based cohort in Rotterdam, The Netherlands, including women in early pregnancy who were resident in the study area, delivering between April 2002 - January 2006	Prospective cohort study	5,993	Maternal nutrition	Histology, ultrasound	9
Timmermans	2012	The Netherlands	Embedded in the Generation R study, a population-based cohort in Rotterdam, The Netherlands, including women in early pregnancy who were resident in the study area, delivering between April 2002 - January 2006	Prospective cohort study	3,207	Maternal nutrition	Histology, ultrasound	6
Tsai	2015	USA	Pregnant women at term (> 37 weeks GA) who were undergoing an elective pre-labour caesarean section at Kapiolani medical center for women and children (Honolulu, Hawaii), August - December 2012	Case-Control	84	Maternal body weight	Histology, biomarker	3
Uhl	2015	Germany, Spain	Participants of the observational PREOBE study, enrolled between 12 - 20 weeks GA at the university of Granada	Case-Control	48	Maternal body weight	Histology	2

Supplemental table 1, continued

Study	Year	Country	Study population	Study design	Sample size	Maternal exposure(s)	Outcome(s)	Quality score*
van Oppenraaij	2012	The Netherlands	Placentas of women scheduled for a legal termination of a viable first trimester pregnancy for social indications at the center of sexual health, Amsterdam, January - October 2009	Case-Control	26	Maternal smoking	Histology	3
Vuorela	2002	Finland	Pregnant women at the Helsinki university central hospital, recruited from a special outpatient clinic for pregnant alcohol and drugs abusers	Prospective cohort study	82	Maternal alcohol use	Biomarker	5
Wallace	2014	UK	Women registered in the Aberdeen maternity and neonatal databank who had their first-ever and second consecutive births at Aberdeen maternity hospital, who booked before 24 weeks GA on both occasions, between 1986 – 2007	Retrospective cohort study	12,740	Maternal body weight	Histology	5
Wang	2014	Australia, China, France, UK	Embedded in the Tasmanian Infant Health Survey, a cohort investigating the cause of sudden infant death syndrome in Tasmania, Australia, January 1988 - December 1995	Prospective cohort study	7,945	Maternal alcohol use, Smoking, Body weight	Histology	7
Wills	2010	India, UK	Women included in the Pune maternal nutrition study, living in 6 villages located 40 - 50 kilometers from Pune, India, 1994 – 1996	Prospective cohort study	478	Maternal body weight	Ultrasound	6
Yigiter	2006	Turkey	Turkish women between 11 - 14 weeks GA to undergo screening for Down syndrome at the Marmara university school of medicine, Istanbul, Turkey between 2001 – 2005	Prospective cohort study	1,275	Maternal smoking	Biomarker	5
Zera	2014	USA	Secondary analysis of a cohort study evaluating biomarkers for the diagnosis of preeclampsia, pregnant women below 15 weeks GA were recruited from 3 urban academic medical centers in Boston and Philadelphia, 2006 - 2008	Prospective cohort study	2,399	Maternal smoking, Body weight	Biomarker	6
Zerón	2013	Mexico	Pregnant women in the first trimester of pregnancy, attending the maternal-perinatal hospital 'Monica Pretelini' Toluca, Mexico, March 2009 - December 2010	Prospective cohort study	26	Maternal body weight, Nutrition	Biomarker	4

ErasmusAGE quality score, ranging from 0-10, resulting from the sum of five sub-items (study design, study size, description of methods and outcomes, adjustment for confounders)

BMI = body-mass index; GA = gestational age; UK = United Kingdom; USA = United States of America

Supplemental Table 2. Markers of placental development and function (ultrasound/serum biomarkers/histology) per periconceptional maternal exposure

a. Markers of placental development and function in the first trimester of pregnancy (N=24)

	Ultrasound	Serum biomarkers	Histology
Exposure: periconceptional maternal smoking	**Static:** - Placental volume: =[146] **Dynamic (Doppler):** - UtA RI: ↑[45] - UmbA RI: ↑[45,91] - Vascularization indices: VI, FI and VFI ↓[146]	**Proteomics:** <u>Vascular:</u> - VEGFA: ↑[162] - PlGF: ↑[28,83] - sFlt-1: =[82] <u>Miscellaneous:</u> - β-hCG: ↓[3,5,82] - PAPP-A: ↓[3,5,42,82,205] - IGF-1: ↓[42] **Genomics:** <u>Vascular:</u> - VEGF gene expression: =[90] - PlGF gene expression: ↑[89,90] - sFLT1 gene expression: =[90] <u>Miscellaneous:</u> - TP53 gene expression: ↑[90] - BAX gene expression: ↑[90] - BAX/BCL2 mRNA: ↑[90]	**Maternal:** <u>Oxidative stress:</u> - Premature depletion cytotrophoblasts[61] - Trophoblast apoptosis: ↑[70] <u>Vascular changes:</u> - Total, central and peripheral villous tree volume and vascular volume: =[182] - Central and peripheral villous tree vascular densities: ↑[182] **Fetal:** No results
Exposure: periconceptional maternal alcohol use	No results	**Proteomics:** <u>Vascular:</u> PlGF: =[184]	No results
Exposure: periconceptional maternal nutrition - *Fish intake*	No results	**Proteomics:** <u>Vascular:</u> - PlGF: =[22] - sFlt-1: =[22]	No results
- *Higher vitamin B2, vitamin D, folate, zinc and irons serum levels*	No results	<u>Vascular:</u> PlGF/(sFlt-1 * sEng): ↑[53]	No results
- *Transfat intake*	No results	<u>Vascular:</u> PlGF/(sFlt-1*sEng): ↓[53]	No results

(Continued)	Ultrasound	Serum biomarkers	Histology
- *Intake of multiple micronutrients*	No results	<u>Vascular:</u> PlGF: =[64]	No results
Exposure: higher periconceptional maternal body weight (in kg)	**Static:** - Placental thickness: =[82] - Basal plate surface: =[82] - Placental 2D volume: =[82]	**Proteomics:** <u>Vascular:</u> - PlGF: ↓[201] - sFlt-1: ↑[51] - sFlt-1: ↓[201] - sFlt-1/PlGF: ↑[51] - sEng: ↓[51] <u>Miscellaneous:</u> - PAI-1/PAI-2 ratio: ↑[167] - Uterine natural killer cells (in decidual mucosa): ↓[220] **Genomics:** <u>Miscellaneous:</u> Altered global gene expression[220]	No results

b. Markers of placental development and function in the second trimester of pregnancy (N=16)

	Ultrasound	Serum biomarkers	Histology
Exposure: periconceptional maternal smoking	**Dynamic (Doppler):** - UtA PI: =[142] - Mean blood flow: =[142] - UmbA RI: ↑[91] - UmbA PI: =[142] - Mean blood flow: ↑[142]	**Proteomics:** <u>Vascular:</u> - PlGF: ↑[83,130] - sFlt-1 : = [82,83] - sFlt-1 : ↓[130] - sEng: ↓[83] <u>Miscellaneous:</u> - β-hCG: ↓[82] - PAPP-A: ↓[82,205] - Inhibin A: ↑[82] - AFP: =[82] - Estriol: =[82]	**Maternal:** <u>Oxidative stress:</u> Trophoblast apoptosis: ↑[70] **Fetal:** No results
Exposure: periconceptional maternal alcohol use	No results	**Proteomics:** <u>Vascular:</u> PlGF: ↑[184]	No results
Exposure: periconceptional maternal nutrition - *Fish intake*	**Dynamic (Doppler):** - UtA PI: ↓[173] - UtA RI: ↓[173] - UmbA PI: ↓[173]	No results	No results
- *Higher serum fatty acid levels*	No results	**Proteomics:** <u>Vascular:</u> - PlGF: ↓[22] - sFlt-1: ↓[22]	No results
- *Intake of multiple micronutrients*	**Dynamic (Doppler):** - UtA PI: ↓[207] - UtA RI: ↓[207] - UmbA PI: =[207] - MCA peak systolic velocity: ↓[207]	<u>Miscellaneous:</u> - β-hCG levels: =[207] - hPL levels: =[207] - PAI-1/PAI-2 ratio: =[207]	No results
Exposure: higher periconceptional maternal body weight (in kg)	**Static:** - 2D placental volume: =[82]	**Proteomics:** <u>Vascular:</u> - PlGF: ↓[130,201]	No results

(Continued)	Ultrasound	Serum biomarkers	Histology
Exposure: higher periconceptional maternal body weight (in kg)	**Static:** - 2D placental volume: ↑[92,170] - Placental thickness: =[82] - Basal plate surface: =[82]	**Proteomics:** <u>Vascular:</u> - sFlt-1: ↑ [51] - sFlt-1: ↓[130,201] - sFlt-1/PlGF ratio: ↑ [51] <u>Miscellaneous:</u> - Placental GH: ↓[43] - sEng: ↓[51,130] - PAI-1/PAI-2: =[167]	No results

c. Markers of placental development and function in the third trimester of pregnancy or at birth (N=61)

	Ultrasound	Serum biomarkers	Metric	Histology
Exposure to: periconceptional maternal smoking	No results	**Proteomics:** Miscellaneous: PAPP-A: ↓[205] **Genomics:** Vascular: - Coagulation genes: ↑[34] - Endothelial genes: ↑[34] - Vascular genes: ↑[34] Inflammatory: Inflammation genes: ↑[34] Miscellaneous: - Xenobiotic genes: ↑[34] - Collagen genes: ↑[34] - Extracellular matrix genes: ↑[34] - Adipocyte genes: ↓[34] - Anion transport genes: ↓[34] - Polyamine biosynthesis genes: ↓[34] - Pregnancy maintenance genes: ↓[34] - Genotoxic stress genes: ↓[34] Miscellaneous: - Placental methylation profiles different[218]	- Placental weight:↓[100,116,134,190,219] - Placental weight: ↑[116,127,171] - Placental weight: =[6,142,213] - Placenta-to-birth weight ratio: ↓[190]	**Maternal:** Oxidative stress: Trophoblast oxygen conductance and resistance: =[37] Miscellaneous: - Villous number: ↓[6] - Vasculosyncytial membrane thickening: ↑[6] - Volume intervillous space: ↑[36] - Trophoblast apoptosis: ↓[70] - Echogenic areas and indentations in chorionic plate: ↑[142] - Placental pathology: =[213] **Fetal:** Oxidative stress: Trophoblast oxygen conductance and resistance: =[3] Vascular changes: - Capillary surface areas: ↑[3] - Capillary volumes: ↓[36] Miscellaneous: Chorangiosis: ↑[6]
- *Periconceptional maternal second hand smoking*	No results	No results	- Placental weight: ↓[134]	No results
Exposure to: periconceptional maternal alcohol use	No results	**Proteomics:** Vascular: PlGF: ↑[184]	- Placental weight: ↓[171,213] - Placenta-to-birth weight ratio: ↓[190,213]	**Maternal:** Vascular changes: Placental hemorrhage: ↑[213]

(Continued)	Ultrasound	Serum biomarkers	Metric	Histology
Exposure to: periconceptional maternal alcohol use				**Maternal:** Inflammatory changes: - Chorioamnionitis: =[213] - Villitis: =[213] - Deciduitis: =[213] - Maternal vascular underperfusion: =[213] Miscellaneous: Accelerated villous maturation: ↑[213]
Exposure to: periconceptional maternal nutrition - *Folic acid use*	**Dynamic (Doppler):** *Folic acid use* - UtA PI: ↓[173] - UtA RI : ↓[173] - UmbA PI↓[173]	No results	- Placental weight: ↑[172] - Ratio placental/fetal weight: =[172]	No results
- *Multiple micronutrients supplementation*	**Dynamic (Doppler):** - UtA RI: ↓[207]	**Proteomics:** Vascular: PlGF: =[64] Miscellaneous: hPL: =[64]	Placental weight: =[102,207]	No results
- *Lower adherence to Mediterranean diet*	No results	No results	Placental weight: ↓[174]	No results
- *Higher adherence to Mediterranean diet*	**Dynamic (Doppler):** - UtA PI: =[174] - UmbA PI=[174]	No results	No results	No results
- *Higher PUFA intake*	No results	No results	Placental weight: ↓[118]	No results
- *Higher protein intake*	No results	No results	Placental weight: ↑[132]	No results
- *Higher dairy intake*	No results	No results	Placental weight: ↑[208]	No results
- *Famine in utero*	No results	No results	Placental length, breadth, thickness, area and volume: ↓[151]	No results
Exposure to: periconceptional maternal body weight (in kg) - *Lower*	No results	No results	- Placental weight: ↓[78,116,122]	**Fetal:** Miscellaneous: Membrane fibrin deposition: ↑[78]

(Continued)	Ultrasound	Serum biomarkers	Metric	Histology
Exposure to: periconceptional maternal body weight (in kg) - *Higher*	(see above)	**Proteomics:** <u>Vascular:</u> - PlGF: ↓[201] - sFlt-1: ↓[201] - VEGF family secretion: =[104] <u>Miscellaneous:</u> - Placental GH: ↓[43] - Placental TauT activity: ↓[1,48] - Placental TauT expression: =[48] - Protein expression: ↑[49] - FABP3 protein expression: ↓[49] - LPL protein: =[49] - LPL activity ↑[49] - PAPPRA/PAPPD/PAPPG protein: =[49] - cIAP1 and cIAP2 protein expression: ↑[103] - FGFR2 secretion: =[104] - sEng secretion: =[104] - Adhesion molecules secretion: =[104] <u>Miscellaneous:</u> - TSPO protein expression: ↓[107] - PAI-1/PAI-2: =[167] - Leptin mRNA: =[175] **Genomics:** <u>Oxidative stress:</u> - miR-210: ↑[133(female fetuses),221] <u>Vascular:</u> - VEGF family gene expression: =[104] - Altered expression angiogenesis genes[153] <u>Miscellaneous:</u> - FABP1 mRNA: ↓[49] - LPL mRNA: =[49] - PAPPRA/PAPPD/PAPPG mRNA: =[49] - cIAP1 and cIAP2 gene expression: ↑[103] - FGFR1/2 mRNA expression: =[104]	*Maternal* - Placental weight: ↑[20,47,49,63,74,78,100, 103,110,116,122, 123,127,171,175,190,194,211,224] - Placental weight: =[21,48,153] - Placental thickness: ↑[20,123] - Ratio placental/fetal weight: ↓[78] - Ratio placental/fetal weight: ↑[190,211] - Ratio fetal/placental weight: ↓[123] *Paternal* - Placental weight: ↑[100] - Placental weight: =[194]	**Maternal:** <u>Vascular changes:</u> - Intervillous thrombi in parenchyma: ↑[74] - Vascular lesions: ↑[78] - Vascular lesions: =[21,112] <u>Inflammatory changes:</u> - Inflammation: ↑[21,40,74] - Inflammation: =[112] <u>Miscellaneous:</u> - Proliferative and apoptotic index: ↓[2] - Chorionic plate area: ↑[20] - Villous lesions: ↑[78] - Immaturity villous tree: ↑[112] - Leptin in vascular endothelial cells: ↑[175] - Leptin in syncytiotrophoblast: =[175] - Leptin receptors in syncytiotrophoblast and intravillous macrophages: =[175] **Fetal:** <u>Vascular changes:</u> - Abnormal spiral artery remodeling: ↑[12] - Vascular lesions: =[21] - Thrombi in chorionic vessels: =[74] - Avascular villi: =[74] <u>Inflammatory changes:</u> - Inflammation: ↑[74] - Neutrophiles infiltration: ↑[78] <u>Miscellaneous:</u> - Marginal insertion umbilical cord: ↑[74] - Chorangiosis: =[74] - Meconium fetal membrane: ↑[78]

(Continued)	Ultrasound	Serum biomarkers	Metric	Histology
Exposure to: periconceptional maternal body weight (in kg) - *Higher*	(see above)	Miscellaneous: - sEng mRNA expression: =[104] - Adhesion molecules mRNA expression: =[104] - Mitochondrial density: =[107] - Cholesterol density: ↓[107] - TSPO gene expression: ↓[107] - Altered expression lipid metabolism genes[153] - Altered expression hormone activity genes[153] - Altered expression cytokine activity genes[153] - Altered expression 72 genes[204] - BDNF mRNA and proBDNF: ↓[221](male fetuses) - Phosphorylation TRKB tyrosin 817 and MAPK p38 (male fetuses): ↑[221] - CD36 mRNA: ↑[49] - SLC27A4 mRNA: ↓[49]	(see above)	(see above)
Exposure to: periconceptional maternal body weight (in kg) *Higher*		**Metabolomics:** Oxidative stress: Markers for oxidative stress: ↑[121] Miscellaneous: - Changed placental fatty acid transfer[4] - Triglyceride levels: ↑[121] - SOD expression: ↑[202] - NF-κB expression: ↑[202]		
- *Interpregnancy loss*	No results		Placental weight: ↓[186]	No results
- *Interpregnancy gain*	No results		Placental weight: ↑[186]	No results

2D= two dimensional; AFP= alpha-fetoprotein; BAX= bclr-like protein 4; BCL2= protein encoded by BAX gene; BDNF= brain derived neurotropic factor; β-hCG= beta human chorionic gonadotropin; BMI= body mass index; CD36= cluster of differentiation 36; cIAP= cellular inhibitors of apoptosis; FABP= fatty acid translocase binding protein; FGFR= fibroblast growth factor; FI= flow index; GH= growth hormone; hPL= human placental lactogen; IGF-1= insulin-like growth factor-1; LPL= lipoprotein lipase; MAPK= mitogen-activated protein kinase; MCA= middle cerebral artery; miRNA= micro ribonucleic acid; mRNA= messenger ribonucleic acid; NF-κB= nuclear factor kappa B; PAPP-A= pregnancy-associated plasma protein A; PAPPRA/PAPPD/PAPPG= undescribed; PAI= plasminogen activator inhibitor; PI= pulsatility index; PlGF= placental growth factor; PlGF/(sFlt-1 * sEng) (angiogenic to antiangiogenic ratio); PUFA= polyunsaturated fatty acids; RI= resistance index; sEng= soluble endoglin; sFLT-1= soluble fms-like tyrosine kinase; SLC27A4= subtype of fatty acid translocase; SOD= superoxide dismutase; TauT= taurine transporter protein; TP53= tumor protein p53; TRWB= tropomyosin receptor kinase B; TSPO= mitochondrial translocator protein; UmbA= umbilical artery; UtA= uterine artery; VEGF= vascular endothelial growth factor; VFI= vascularization flow index; VI= vascularization index

Supplemental File 1. Search strategy

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('placenta function'/de OR 'placenta development'/de OR 'placenta circulation'/exp OR 'placenta disorder'/de OR 'placenta weight'/de OR 'placenta protein 14'/de OR (('vasculotropin'/de OR 'microRNA'/exp OR 'prolactin'/exp OR 'pregnancy associated plasma protein A'/de OR 'chorionic gonadotropin beta subunit'/de OR 'vasculotropin receptor 1'/de OR 'vasculotropin receptor'/de) AND ('placenta'/exp)) OR ('organ size'/de AND placenta/de) OR (((placenta* OR uteroplacenta*) NEAR/3 (function* OR dysfunction* OR develop* OR circulation* OR hypocirculation* OR vascul* OR 'blood flow' OR growth OR weight* OR size OR measure* OR morpholog* OR disorder* OR disease* OR ischem* OR ischaem* OR infarct* OR patholog* OR characterist* OR complication* OR abnormal* OR architecture* OR angioarchitecture* OR problem* OR impair* OR 'flow index' OR oxidat* OR protein* OR restrict* OR stress OR oxygen* OR microscop* OR histolog* OR hormone* OR doppler)) OR glycodelin* OR (((vascular NEAR/3 endothel* NEAR/3 growth) OR microRNA OR 'micro RNA' OR mirna* OR vegf OR prolactin OR (pregnan* NEAR/3 associat NEAR/3 protein* NEAR/3 A) OR papp-a OR (chorionic* NEAR/3 gonadotropin* NEAR/3 beta) OR (hcg NEAR/3 beta) OR (vasculotropin* NEAR/3 receptor*) OR flt OR (Fms NEAR/3 tyrosine)) AND (placenta*))):ab,ti) AND ('maternal behavior'/exp OR 'maternal nutrition'/exp OR 'paternal behavior'/exp OR 'parental behavior'/de OR 'parental smoking'/exp OR 'parental stress'/exp OR 'maternal stress'/exp OR 'prenatal drug exposure'/exp OR 'folic acid'/de OR (((maternal* OR mother* OR paternal* OR father* OR parent*) NEAR/3 ('body mass' OR bmi OR overweight OR obes*)) OR ((maternal* OR mother* OR paternal* OR father* OR parent* OR prenatal* OR preconcept* OR pregnan* OR maternal* OR geograph*) NEAR/3 (nutriti* OR undernutriti* OR malnutriti* OR diet* OR behav* OR factor* OR smoking OR smoke OR predict* OR stress* OR alcohol* OR drinking OR 'drug use' OR substance* OR effect OR effects OR pollut* OR tobacco OR caffeine OR cocaine)) OR 'folic acid'):ab,ti) AND ('cohort analysis'/exp OR 'longitudinal study'/exp OR 'prospective study'/exp OR 'retrospective study'/exp OR 'multicenter study'/exp OR 'epidemiological data'/de OR epidemiology/de OR 'observational study'/de OR 'major clinical study'/exp OR 'controlled study'/exp OR 'multivariate analysis'/exp OR 'population research'/exp OR 'predictive value'/exp OR (cohort* OR longitudin* OR prospectiv* OR retrospectiv* OR multicent* OR epidemiolog* OR observation* OR (control* NEAR/3 stud*) OR registry OR registries OR ((correlat* OR associat* OR relat* OR factor* OR population*) NEAR/15 (stud* OR analy* OR investigat* OR determin*)) OR multivariate* OR multiparameter* OR (multi NEXT/1 (variate* OR parameter*)) OR chart* OR predict*):ab,ti) NOT ([animals]/lim NOT [humans]/lim) NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Editorial]/lim) AND [english]/lim

Medline ovid
("placenta"/ab OR "placenta"/ae OR "placenta"/bl OR "placenta"/gd OR "Placenta Diseases"/ OR "Placental Insufficiency"/ OR ("organ size"/ AND placenta/) OR (("Vascular Endothelial Growth Factor A"/ OR "microRNAs"/ OR "prolactin"/ OR "Pregnancy-Associated Plasma Protein-A"/ OR "Chorionic Gonadotropin, beta Subunit, Human"/) AND ("placenta"/)) OR (((placenta* OR uteroplacenta*) ADJ3 (function* OR dysfunction* OR develop* OR circulation* OR hypocirculation* OR vascul* OR "blood flow" OR growth OR weight* OR size OR measure* OR morpholog* OR disorder* OR disease* OR ischem* OR ischaem* OR infarct* OR patholog* OR characterist* OR complication* OR abnormal* OR architecture* OR angioarchitecture* OR problem* OR impair* OR "flow index" OR oxidat* OR protein* OR restrict* OR stress OR oxygen* OR microscop* OR histolog* OR hormone* OR doppler)) OR glycodelin* OR (((vascular ADJ3 endothel* ADJ3 growth) OR microRNA OR "micro RNA" OR mirna* OR vegf OR prolactin OR (pregnan* ADJ3 associat ADJ3 protein* ADJ3 A) OR papp-a OR (chorionic* ADJ3 gonadotropin* ADJ3 beta) OR (hcg ADJ3 beta) OR (vasculotropin* ADJ3 receptor*) OR flt OR (Fms ADJ3 tyrosine)) AND (placenta*))).ab,ti.) AND ("Maternal Behavior"/ OR exp "Maternal Nutritional Physiological Phenomena"/ OR "Paternal Behavior"/ OR (exp parents/ AND (smoking/ OR "Stress, Psychological"/)) OR "Prenatal Exposure Delayed Effects"/ OR "folic acid"/ OR (((maternal* OR mother* OR paternal* OR father* OR parent*) ADJ3 ("body mass" OR bmi OR overweight OR obes*)) OR ((maternal* OR mother* OR paternal* OR father* OR parent* OR prenatal* OR preconcept* OR pregnan* OR maternal* OR geograph*) ADJ3 (nutriti* OR undernutriti* OR malnutriti* OR diet* OR behav* OR factor* OR smoking OR smoke OR predict* OR stress* OR alcohol* OR drinking OR "drug use" OR substance* OR effect OR effects OR pollut* OR tobacco OR caffeine OR cocaine)) OR "folic acid").ab,ti.) AND (exp "Cohort Studies"/ OR "multicenter study"/ OR "Epidemiological Monitoring"/ OR "Epidemiologic Methods"/ OR epidemiology.xs. OR epidemiology/ OR "observational study"/ OR exp "Clinical Trial"/ OR "Multivariate Analysis"/ OR (cohort* OR longitudin* OR prospectiv* OR retrospectiv* OR multicent* OR epidemiolog* OR observation* OR (control* ADJ3 stud*) OR registry OR registries OR ((correlat* OR associat* OR relat* OR factor* OR population*) ADJ15 (stud* OR analy* OR investigat* OR determin*)) OR multivariate* OR multiparameter* OR (multi ADJ (variate* OR parameter*)) OR chart* OR predict*).ab,ti.) NOT (exp animals/ NOT humans/) NOT (letter OR news OR comment OR editorial OR congresses OR abstracts).pt. AND english.la.

Cochrane
(((((placenta* OR uteroplacenta*) NEAR/3 (function* OR dysfunction* OR develop* OR circulation* OR hypocirculation* OR vascul* OR 'blood flow' OR growth OR weight* OR size OR measure* OR morpholog* OR disorder* OR disease* OR ischem* OR ischaem* OR infarct* OR patholog* OR characterist* OR complication* OR abnormal* OR architecture* OR angioarchitecture*

OR problem* OR impair* OR 'flow index' OR oxidat* OR protein* OR restrict* OR stress OR oxygen* OR microscop* OR histolog* OR hormone* OR doppler)) OR glycodelin* OR (((vascular NEAR/3 endothel* NEAR/3 growth) OR microRNA OR 'micro RNA' OR mirna* OR vegf OR prolactin OR (pregnan* NEAR/3 associat NEAR/3 protein* NEAR/3 A) OR papp-a OR (chorionic* NEAR/3 gonadotropin* NEAR/3 beta) OR (hcg NEAR/3 beta) OR (vasculotropin* NEAR/3 receptor*) OR flt OR (Fms NEAR/3 tyrosine)) AND (placenta*))):ab,ti) AND ((((maternal* OR mother* OR paternal* OR father* OR parent*) NEAR/3 ('body mass' OR bmi OR overweight OR obes*))   OR ((maternal* OR mother* OR paternal* OR father* OR parent* OR prenatal* OR preconcept* OR pregnan* OR maternal* OR geograph*) NEAR/3 (nutriti* OR undernutriti* OR malnutriti* OR diet* OR behav* OR factor* OR smoking OR smoke OR predict* OR stress* OR alcohol* OR drinking OR 'drug use' OR substance* OR effect OR effects OR pollut* OR tobacco OR caffeine OR cocaine)) OR 'folic acid'):ab,ti)

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TS=(((((placenta* OR uteroplacenta*) NEAR/2 (function* OR dysfunction* OR develop* OR circulation* OR hypocirculation* OR vascul* OR "blood flow" OR growth OR weight* OR size OR measure* OR morpholog* OR disorder* OR disease* OR ischem* OR ischaem* OR infarct* OR patholog* OR characterist* OR complication* OR abnormal* OR architecture* OR angioarchitecture* OR problem* OR impair* OR "flow index" OR oxidat* OR protein* OR restrict* OR stress OR oxygen* OR microscop* OR histolog* OR hormone* OR doppler)) OR glycodelin* OR (((vascular NEAR/2 endothel* NEAR/2 growth) OR microRNA OR "micro RNA" OR mirna* OR vegf OR prolactin OR (pregnan* NEAR/2 associat NEAR/2 protein* NEAR/2 A) OR papp-a OR (chorionic* NEAR/2 gonadotropin* NEAR/2 beta) OR (hcg NEAR/2 beta) OR (vasculotropin* NEAR/2 receptor*) OR flt OR (Fms NEAR/2 tyrosine)) AND (placenta*)))) AND ((((maternal* OR mother* OR paternal* OR father* OR parent*) NEAR/2 ("body mass" OR bmi OR overweight OR obes*)) OR ((maternal* OR mother* OR paternal* OR father* OR parent* OR prenatal* OR preconcept* OR pregnan* OR maternal* OR geograph*) NEAR/2 (nutriti* OR undernutriti* OR malnutriti* OR diet* OR behav* OR factor* OR smoking OR smoke OR predict* OR stress* OR alcohol* OR drinking OR "drug use" OR substance* OR effect OR effects OR pollut* OR tobacco OR caffeine OR cocaine)) OR "folic acid")) AND ((cohort* OR longitudin* OR prospectiv* OR retrospectiv* OR multicent* OR epidemiolog* OR observation* OR (control* NEAR/2 stud*) OR registry OR registries OR ((correlat* OR associat* OR relat* OR factor* OR population*) NEAR/15 (stud* OR analy* OR investigat* OR determin*)) OR multivariate* OR multiparameter* OR (multi NEAR/1 (variate* OR parameter*)) OR chart* OR predict*)) NOT ((animal* OR rat OR rats OR mouse OR mice OR murine OR nonhuman* OR primate* OR rabbit* OR pig OR pigs OR porcine OR ovine OR sheep OR cattle OR cow OR cows OR bovine OR mare OR mares OR horse* OR equus OR equine) NOT (human* OR animal*))) AND LA=(english) AND DT=(article)

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"placenta|placental development|circulation|weight|size|insufficiency" "maternal|paternal|parental nutrition|factor|factors|bmi|overweight|diet|behavior|smoking|stress|drinking|substance cohort|longitudinal|prospective|retrospective|epidemiology

Supplemental File 2. ErasmusAGE quality score

Adjusted quality score, version 03-04-2017. Original: ErasmusAGE, 24 June 2013.

This quality score can be used to assess the quality of studies included in systematic reviews and meta-analyses and is applicable to both interventional and observational studies. The score was designed based on previously published scoring systems (Carter et al, 2010 and the Quality Assessment Tool for Quantitative Studies*)*. The quality score is composed of 5 items, and each item is allocated 0, 1 or 2 points. This allows a total score between 0 and 10 points, 10 representing the highest quality.

The version presented below is a general version and needs to be adapted for each review separately, e.g. concerning what study size is large or small within the study field, what exposure and outcome measurement methods are adequate, and what the key confounders are. Decisions on these detailed criteria should be based on literature, guidelines and/or discussions with experts. The criteria should be defined before the review process.

1. **Study design**
0 for studies with cross-sectional data collection
1 for studies with longitudinal data collection (both retrospective and prospective)
2 for intervention studies

2. **Study size** (predefined)*
Observational studies
0 small population for analysis: n= <100
1 intermediate population for analysis: n= 100-500
2 large population for analysis: n= >500

Intervention studies
0 small population for analysis: n= <50
1 intermediate population for analysis: n= 50-100
2 large population for analysis: n= >100

3. **Exposure**
Observational studies
0 if the study used no appropriate exposure measurement method (or if not reported)
1 if the study used moderate quality exposure measurement methods (not clearly defined, use of inappropriate questionnaires or no multiple-day records of exposure) for one of the following items:
- *maternal smoking*
- *maternal alcohol use*
- *maternal caffeine intake*
- *maternal nutrition*
- *maternal body weight*
2 if the study used adequate exposure measurement methods (clearly defined, use of appropriate questionnaires, objective measurements or multiple-day records of exposure) for one of the following items:
- *maternal smoking*
- *maternal alcohol use*
- *maternal caffeine intake*
- *maternal nutrition*
- *maternal body weight*

Intervention studies
0 if the intervention was not described or not blinded
1 if the intervention was adequately single blinded.
2 if the intervention was adequately double-blinded.

4. **Outcome**
0 if the study used no appropriate outcome measurement method of placental development and/or function or if not reported
1 if the study used an appropriate outcome measurement of placental development and/or function, but without adequate description of the method, for one of the following outcomes as mentioned below*

- placental ultrasound
- placental biomarkers
- placental histology
2 if the study used an appropriate outcome measurement of placental development and/or function and adequately described the method of assessment for one of the following outcomes as mentioned below*
- placental ultrasound
- placental biomarkers
- placental histology

5. Adjustments

0 if findings are not controlled for at least for all three key confounders, as mentioned below† *
1 if findings are controlled for key confounders
- maternal age
- maternal ethnicity
- maternal socio-economic status
2 if an intervention is adequately randomized <u>or</u> when findings are additionally controlled for at least two of the following additional covariates
- birth weight
- gestational age at delivery
- parity
- (any) maternal disease with obstetrical implications
- infant sex
- other environmental modifiable exposures

* Needs to be specified for each review, based on literature, guidelines and/or expert opinions in the field
† Either adjusted for in the statistical analyses; stratified for in the analyses; or not applicable (e.g. a study in women only does not require controlling for sex)

References

1. Carter P, Gray LJ, Troughton J, Khunti K, Davies MJ. Fruit and vegetable intake and incidence of type 2 diabetes mellitus: systematic review and meta-analysis. *BMJ.* 2010;341:c4229.
2. National Collaborating Centre for Methods and Tools (2008). Quality Assessment Tool for Quantitative Studies. Hamilton, ON: McMaster University. (Updated 13 April, 2010).

CHAPTER 3

New imaging markers for preconceptional and first-trimester utero-placental vascularization

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ABSTRACT

Introduction: The availability of imaging makers of early placental circulation development is limited. This study aims to develop a feasible and reliable method to assess preconceptional and early first-trimester utero-placental vascular volumes using three-dimensional power Doppler (3D PD) ultrasound on two different Virtual Reality (VR) systems.

Methods: 3D PD ultrasound images of the uterine and placental vasculature were obtained in 35 women, either preconceptionally (n=5), or during pregnancy at 7 (n=10), 9 (n=10) or 11 (n=10) weeks of gestation. Preconceptional uterine vascular volume (UVV), first-trimester placental vascular volume (PVV) and embryonic vascular volume (EVV) were measured by two observers on two VR systems, i.e., a Barco I-Space and VR desktop. Intra- and inter-observer agreement and intersystem agreement were assessed by intra-class correlation coefficients (ICC) and absolute and relative differences.

Results: Uterine-, embryonic- and placental vascular volume measurements showed good to excellent intra- and inter-observer agreement and inter-system reproducibility with most ICC above 0.80 and relative differences of less than 20% preconceptionally and almost throughout the entire gestational age range. Inter-observer agreement of PVV at 11 weeks gestation was suboptimal (ICC 0.69, relative difference 50.1%).

Discussion: Preconceptional and first-trimester 3D PD ultrasound utero-placental and embryonic vascular volume measurements using VR are feasible and reliable. Longitudinal cohort studies with repeated measurements are needed to further validate this and assess their value as new imaging markers for placental vascular development and ultimately for the prediction of placenta-related pregnancy complications.

INTRODUCTION

Worldwide, millions of women develop fertility problems or placenta-related pregnancy complications, such as pregnancy-induced hypertension, preeclampsia, fetal growth restriction and preterm birth every year. These complications not only affect the outcome of a pregnancy, but some can also impact the health of the mother and her offspring later in life[1-3].

These problems in reproduction can be due to derangements in the utero-placental vascularization and originate during the periconception period, i.e., 14 weeks prior to conception until 10 weeks thereafter[4]. Preconceptional uterine vascularization is involved in endometrial receptivity, decidual selectivity and subsequent implantation in combination with complex interactions between hormones, nutrients, growth factors and endometrial genes[5,6]. A decreased (sub)endometrial blood flow has been associated with decreased pregnancy rates[7,8].

Human placentation is characterized by the remodeling of the uterine circulation, in particular of the spiral arteries. Remodeling optimizes maternal blood distribution through a low-resistance uterine vascular network and ultimately into the placental intervillous chamber[9]. Up to around 9 weeks of gestation, extravillous trophoblast plugs limit maternal blood entry into these intervillous chambers. These plugs disintegrate thereafter, resulting in the onset of the utero-placental circulation[10]. An imbalance in this delicate phenomenon is hypothesized to be the principal mechanism leading to early pregnancy failure. Similarly, if the uterine portion of the utero-placental circulation fails to develop, adequate placental and fetal growth will fail[9].

Doppler ultrasound imaging and maternal serum biomarkers of placenta function such as placental growth factor (PlGF) or pregnancy-associated plasma protein-A (PAPP-A) have been used to investigate abnormal placentation[11]. Availability of real-time imaging markers for assessment of *in vivo,* early uterine and placental vascularization and function remains limited. The current state-of-the-art technology for evaluation of *in utero* placental vasculature morphology is three-dimensional power Doppler (3D PD) ultrasound[12]. So far, 3D vascular volumes can be assessed using the Virtual Organ Computer-aided AnaLysis (VOCAL) tool to quantify placental vascularization through calculation of vascularization indices (VI), flow indices (FI) and vascularization-flow indices (VFI). However, results regarding reproducibility are conflicting[12-14]. Variations in ultrasound machine settings and also distances between the range of interest and ultrasound transducer influence VOCAL vascularization indices, by affecting power Doppler calculations. Furthermore, despite availability of 3D volumetric data, measurements are still performed in a two-dimensional (2D) plane, and consequently the third dimension that allows for more precise and volume measurements is not used. At the Erasmus MC, we have developed a novel, innovative application, called V-Scope, that displays volumetric ultrasound datasets as holograms, using the Barco I-Space CAVE™-like virtual reality (VR) system (Barco NV, Belgium)[15,16]. Recently, a VR desktop system, based on technical principles of the I-Space, was developed to enable clinical implementation of VR[17]. So far, studies using VR showed accurate and reproducible embryonic and brain development measurements in early pregnancy and utero-placental measurements in the late first trimester of pregnancy[18-21].

The aim of this study is to assess feasibility and reliability of 3D PD ultrasound in combination with two VR systems (I-Space and VR desktop) to measure preconceptional and first-trimester vascular volumes of the uterus, placenta and embryo.

METHODS

Study design

Preconceptional, 5 women undergoing *in vitro* fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI) treatment were recruited from the Department of Reproductive Medicine. Two 3D PD ultrasound images of the utero-placental vasculature were obtained before ovum pick-up. Further, this study was embedded in the prospective, tertiary hospital-based Rotterdam periconception cohort (Predict study) with a focus on the influence of periconceptional lifestyle and environmental factors on human embryonic and fetal growth and development[22]. In 30 pregnant Predict study participants, two 3D PD ultrasound utero-placental vascularization images were obtained in the first trimester of pregnancy, i.e., either at 7 (*n=10*), 9 (*n=10*) or 11 (*n=10*) weeks gestational age (GA). Women at least 18 years of age and prior to pregnancy or with a singleton pregnancy were eligible for inclusion. GA was calculated from the first day of the last menstrual period (LMP) in spontaneous pregnancies, or from oocyte pick-up day plus 14 days in IVF/ICSI pregnancies. In pregnancies originating from cryopreserved embryo transfer it was calculated from the transfer day plus 17 or 18 days, depending on the number of days between oocyte pick-up and embryonic cryopreservation. In regular menstrual cycles, but more than 3 days different from 28 days, we adjusted GA for cycle duration. If the LMP was unknown or GA determined by crown-rump length (CRL) differed more than 7 days from the LMP, GA was based on CRL[22]. Study protocols were approved by the Erasmus MC medical ethics review board and written informed consent was obtained from all participants (MEC 2004-227 and METC 2015-494).

Ultrasound scans

Ultrasound scans were performed by one experienced sonographer (IR) using a Voluson Expert E8 or E10 system (GE, Zipf, Austria) ultrasound machine with standard settings (pulse repetition frequency 0.6 kHz, wall motion filter 'low1', quality 'high', gain adjusted to individual image characteristics), using a 6-12 MHz transvaginal probe. To minimize artifacts and measurement errors by movement, participants were asked to hold their breath for approximately 30 seconds during image acquisition. As variations in uterine position require individual adaptions to optimize image acquisition, two ultrasound volumes were obtained per participant. The first volume was acquired visualizing the uterus in the midsagittal plane. The second volume was obtained after turning the ultrasound transducer 90 degrees perpendicular to the first position. All ultrasound examinations were performed according to international guidelines on safe use of Doppler ultrasound in the first trimester of pregnancy and as such, total scanning time was kept as low as possible (ALARA-principle) and always <30 minutes to avoid unnecessary exposure[23-25]. The

settings during 3D PD ultrasound use resulted in average power levels (i.e. thermal index <0.7) theoretically allowing for unlimited scanning time. However, 3D PD ultrasound use was limited to averagely one minute (two times a volume acquisition of 30 seconds).

Virtual Reality technique

In the Barco I-Space, a CAVE™-like VR environment, using the V-Scope volume rendering application, 3D ultrasound volumes can be visualized as true 3D "holograms"[16]. Additional depth perception of VR enables better visualization and thus assessment of the utero-placental vascularization. Also, 3D interaction makes accurate volumetric measurements feasible. To enable future clinical implementation of VR, a VR desktop system, using the same V-Scope software, was developed and validated by using the I-Space as reference standard[17]. The VR desktop consists of a personal computer with V-Scope software, a 2D monitor displaying the user interface for selecting the measurement tools, a 3D monitor to display the 3D volume, a tracking system for observer interaction with the 3D volume, a pair of stereoscopic glasses to obtain depth perception and a six degrees-of-freedom mouse for 3D volume manipulation[17].

Detailed measurements in the I-Space and on the VR desktop were performed by two researchers according to a standard protocol. Semi-automatic volume measurements of the utero-placental vasculature were obtained by thresholding the 8-bit (range 0-255) Doppler magnitude data. As previously published, the lower-Doppler threshold level was set at a value of 100. This means that only voxels with a Doppler value of 100 or higher are colored and counted by semi-automatic calculations. This enabled the most optimal visualization of the utero-placental vasculature[20,21].

To measure the uterine vasculature in preconceptional ultrasound volumes, we used differences in echogenicity of the uterine and surrounding tissues. Preconceptional uterine vascular volumes (UVV) were calculated after removing artifacts, recognizable by their stripe-like appearance, with a virtual eraser, i.e. deselecting voxels that were initially selected by the thresholding step. In pregnancy, differences in grey values between placental and myometrial tissue were used to selectively measure placental vasculature. By removing grey values and vessels up to the placenta, only placental blood spaces were measured using semi-automated calculations. The obtained total vascular volume (TVV) was calculated after removing artifacts. Thereafter, the embryonic vascular volume (EVV) was identified and measured by erasing selected embryonic vascular structures and calculating the difference with previously obtained TVV. Finally, to select the placental vascular volume (PVV) only, grey values in the volume were used to identify surrounding myometrial tissues. The complete myometrium was then erased using the virtual brush to the margin of the placental tissue interface, leaving the (PVV) (**Figure 1**)[20]. At this stage, it is not possible with VR technology to make a distinction between the maternal blood space and embryonic vasculature within the placental vascular volume.

In VR, the quality of both acquired 3D PD ultrasound volumes at each time point, was scored based on presence of artifacts due to maternal and/or embryonic movements (yes/no), presence of acoustic shadowing (yes/no), volume completeness (complete/incomplete), placental position in relation to the transducer (far/close) and overall quality (low/average/good). The volume with

the highest score or, in cases of equal scores, the first volume was used for further analysis. **Supplemental Figure 1** displays a flowchart of the ultrasound volume acquisition, the VR measurements and the comparisons used to assess agreement between two trained observers (IR and AF) and the two VR systems. For intra- and inter-observer reliability, each observer measured the highest quality 3D PD ultrasound volume twice on different days in the I-Space. The same steps were repeated on the VR desktop. The observers performed their measurements independently, and were blinded to each other's results.

Statistical analysis

Because of skewed distributions, vascular volumes were log transformed prior to analysis. The I-Space system was considered the reference standard when determining intra-and inter-observer as well as VR intersystem reproducibility. Scatterplots with corresponding Pearson's correlation coefficients (R-values) were used to depict correlations between individual measurements for utero-placental vascular volumes. To quantify intra- and inter-observer reproducibility, intra-class correlation coefficients (ICC) were calculated per time point of ultrasound acquisition. Good agreement was defined as an ICC of 0.80 or higher. The mean of the absolute differences, i.e., the difference between first and second measurement, and the relative differences, i.e., the difference between natural log values of the first and second measurement divided by the mean natural log values of the two measurements, of the measurements with corresponding 95% confidence intervals were also calculated. Bland-Altman plots were composed to assess agreement between the two VR systems, displaying mean differences between utero-placental vascular measurements with corresponding 95% limits of agreement. Analyses were performed using SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA), *p*-values <0.05 were considered statistically significant.

Figure 1. Three-dimensional power Doppler ultrasound images of utero-placental vascular volumes preconceptional and in pregnancy at 9 weeks GA visualized by Virtual Reality (VR)
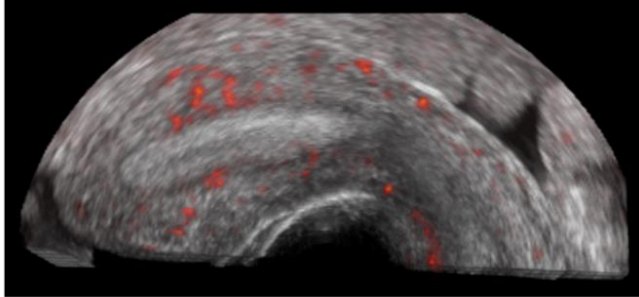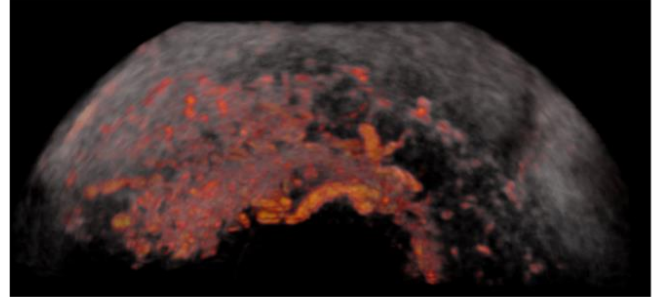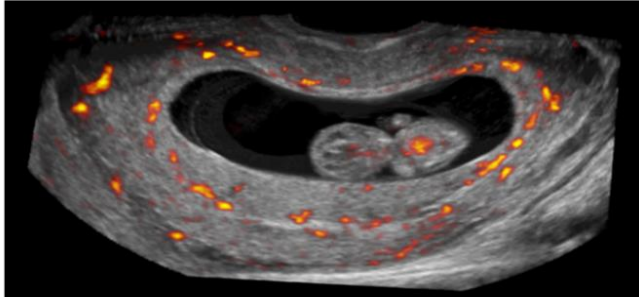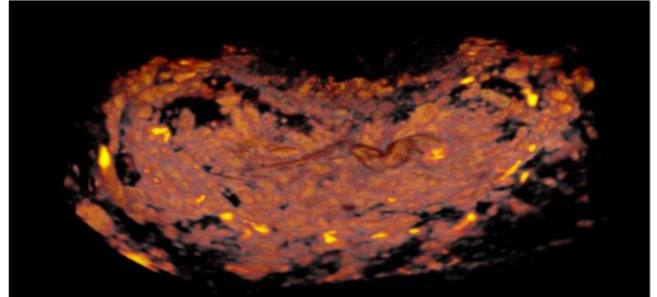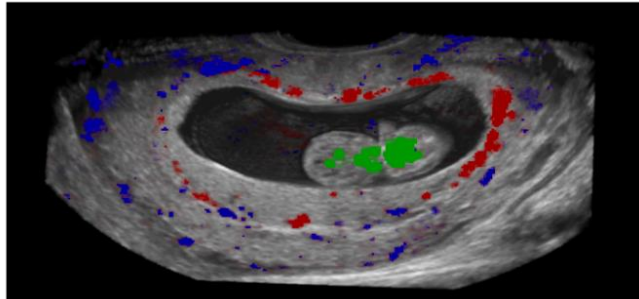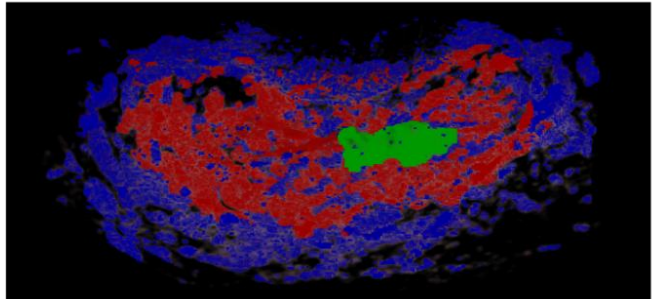

a: Slice view of a midsagittal uterine section showing the preconceptional uterine vascular volume (UVV, orange), with surrounding grey values representing the uterine tissue

b: Volume view of figure 1a showing only the preconceptional uterine vascular volume (UVV, orange), after setting a threshold for grey values

c: Slice view of a total obtained utero-placental vascular volume in pregnancy, with surrounding grey values representing the uterine tissue

d: Volume view of figure 1c showing the total obtained utero-placental vascular volume in pregnancy, after setting a threshold for grey values

e: Slice view of a total obtained utero-placental vascular volume in pregnancy, with surrounding grey values representing the uterine tissue and marked vessel subtypes (uterine vessels (blue); placental vessels (PVV, red); embryonic vessels (EVV, green)

f: Volume view of figure 1e, showing marked vessel subtypes (uterine vascular volume (blue); placental vascular volume (PVV, red); embryonic vascular volume (EVV, green))

RESULTS

The median age of all women (*n*=35) was 33 years (range: 26-41) and median body mass index (BMI) was 23.0 kg/cm² (range: 19-35). Seventeen women (49%) were nulliparous. All preconceptional women (*n*=5) were undergoing IVF/ICSI treatment. Of the 30 pregnant women, 14 (47%) conceived after IVF/ICSI. In pregnancy, we observed higher median utero-placental vascular volumes with advancing GA.

In total, 70 vascular volume measurements were acquired. Quality was good in 31 volumes, average in 35 volumes and low in 4 volumes. All volumes were eligible for further vascular volume measurements. **Table 1** provides an overview of median vascular volumes in cm³ and intra- and inter-observer agreement of utero-placental vascular measurements using the I-space. Preconceptional, intra- and inter-observer agreement for UVV was excellent with ICC ≥0.93. In pregnancy, ICC for intra- and inter-observer agreement for utero-placental vascular volumes ranged from 0.69-0.99 for the entire GA range. Mean relative differences for all utero-placental vascular volumes were below 20%, except for inter-observer agreement of PVV at 7 and 11 weeks GA with a relative mean difference of 20.5% and 50.1%, respectively.

Supplemental Table 1 provides an overview of median vascular volumes and the intra- and inter-observer agreement of utero-placental vascular measurements using the VR desktop. Intra- and inter-observer agreement for preconceptional UVV was excellent with ICC ≥0.92. In pregnancy, ICC for intra- and inter-observer agreement for utero-placental vascular volumes ranged from 0.63-0.99 for the entire GA range. Mean relative differences for all utero-placental vascular volumes were below 20%, except for inter-observer agreement of EVV at 7 weeks GA of -36.7% and PVV at 7 and 11 weeks GA with a relative mean difference of 21.5% and 21.4% respectively.

Table 2 shows the agreement of utero-placental vascular measurements between the two VR systems preconceptionally and in pregnancy. With ICC >0.85, all utero-placental vascular volume measurements show good to excellent intersystem agreement. Mean relative differences for all utero-placental vascular volumes were below 20%. **Supplemental Figure 2** shows strong correlations (R=0.89-1.00) of utero-placental vascular measurements between the two VR systems. **Figure 2** shows the Bland-Altman plots for relative differences of utero-placental vascular measurements between the VR systems, with mean relative differences below 20%.

Table 1. Measurements and intra- and inter-observer reliability parameters of utero-placental vascular volumes using the I-space Virtual Reality system

| | | | | | Intra-observer variability | | | | Inter-observer variability | | | |
		Median [range] (cm^3)		ICC	Mean difference (cm^3) [+ 95% CI]		Relative mean difference (%) [+ 95% CI]		ICC	Mean difference (cm^3) [+ 95% CI]		Relative mean difference (%) [+ 95% CI]	
Preconception *(N=5)*	**UVV**	1.80	[0.97;5.52]	0.97	0.41	[-0.01;0.84]	14.2	[8.0;14.2]	0.93	-0.27	[-0.55;0.01]	-0.27	[-0.55;0.01]
Pregnancy													
7 weeks GA	**PVV**	0.37	[0.10;1.04]	0.97	0.03	[-0.01;0.07]	4.0	[-7.3;15.4]	0.88	0.00	[-0.16;0.16]	0.00	[-0.16;0.16]
(N=10)	**EVV**	0.04	[0.01;0.18]	0.97	0.00	[0.00;0.01]	11.6	[-2.0;25.2]	0.95	-0.01	[-0.02;0.00]	-0.01	[-0.02;0.00]
9 weeks GA	**PVV**	4.48	[1.57;9.16]	0.95	-0.23	[-0.76;0.31]	-3.6	[-15.6;8.5]	0.90	0.62	[-0.16;1.40]	0.62	[-0.16;1.40]
(N=10)	**EVV**	0.44	[0.23;0.78]	0.98	0.00	[-0.02;0.03]	0.8	[-5.0;6.7]	1.00	0.01	[0.00;0.01]	0.01	[0.00;0.01]
11 weeks GA	**PVV**	6.99	[1.90;16.21]	0.94	1.05	[-0.17;2.26]	8.0	[-1.3;18.8]	0.69	2.56	[0.90;4.21]	2.56	[0.90;4.21]
(N=10)	**EVV**	1.34	[0.57;2.20]	0.90	0.16	[-0.05;0.36]	8.8	[-6.6;22.6]	0.99	0.00	[-0.05;0.06]	0.00	[-0.05;0.06]

ICC = intraclass correlation coefficients; CI = confidence interval; GA = gestational age; UVV = uterine vascular volume; PVV = placental vascular volume; EVV = embryonic vascular volume.

Table 2. Inter-system reliability parameters of utero-placental vascular volume measurements using the I-Space and Virtual Reality desktop systems

		ICC	Mean difference (cm³) [+ 95% CI]		Relative mean difference (%) [+ 95% CI]	
Preconception (N=5)	**UVV**	1.00	-0.13	[-0.20;0.06]	-5.5	[-6.8;-4.2]
Pregnancy						
7 weeks GA	**PVV**	0.99	-0.01	[-0.05;0.02]	-0.7	[-7.2;5.8]
(N=10)	**EVV**	0.98	0.00	[-0.01;0.00]	-10.9	[-20.1;-1.8]
9 weeks GA	**PVV**	0.87	0.00	[-0.41;0.75]	8.5	[-9.1;26.0]
(N=10)	**EVV**	0.98	0.17	[-0.02;0.02]	-0.9	[-6.7;4.9]
11 weeks GA	**PVV**	0.93	1.50	[0.14;2.87]	16.5	[1.9;31.1]
(N=10)	**EVV**	0.97	0.06	[-0.02;0.13]	4.9	[-0.4;10.2]

ICC = intraclass correlation coefficients; CI = confidence interval; GA = gestational age; UVV = uterine vascular volume; PVV = placental vascular volume; EVV = embryonic vascular volume.
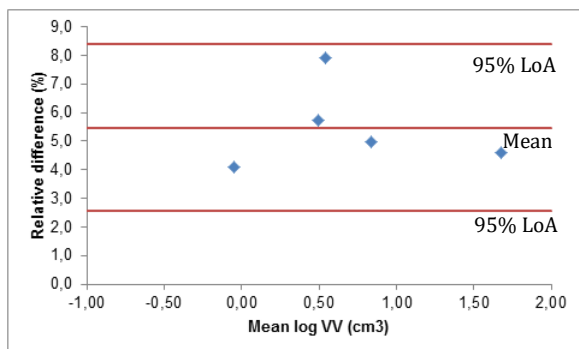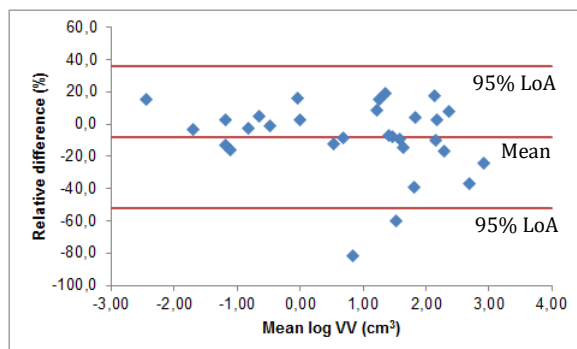
Figure 2. Bland-Altman plots: relative differences in utero-placental vascular volume measurements between two Virtual Reality systems

a. UVV (preconception, N=5)

b. PVV (pregnancy, N=30)

c. EVV (pregnancy, N=30)
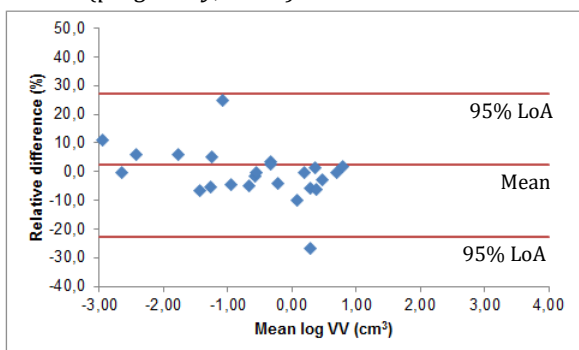
EVV= embryonic vascular volume; GA= gestational age PVV = placental vascular volume; UVV= uterine vascular volume; VV = vascular volume.

Mean= average measurement bias in relative differences; 95% LoA= Upper and lower 95% limit of agreement for relative differences (mean difference ±2SD); Log= natural log transformation of utero-placental vascular volumes to achieve a normal distribution

DISCUSSION

The data of our pilot study indicate that the utero-placental and embryonic circulations can be evaluated reliably and accurately with 3D PD ultrasound and VR techniques. Overall, utero-placental vascular measurements were feasible and reliable with good to excellent intra- and inter-observer agreement. Furthermore, there was excellent VR intersystem agreement with most ICC >0.85 and relative differences <20% both preconceptionally and throughout the entire GA range. Only the PVV at 11 weeks GA measured in the I-Space had a suboptimal inter-observer agreement (ICC 0.69, relative difference 50.1%). This is probably due to a less clear visualization of the boundary between the placenta and underlying uterine tissue at that gestational age. It highlights the possible use of these imaging techniques of assessment of the utero-placental circulation at an early stage of development.

Previous studies have mainly focused on quantification of placental vascularization by using VOCAL to measure VI, FI and VFI, with average to good reproducibility[12-14,20,21]. Differences in machine settings, circumstances for image acquisition (i.e. distance of utero-placental vessels from the transducer either due to patient characteristics or mode of image acquisition (transvaginally or transabdominally)), or relatively small sample sizes could be related to variations in reproducibility. VOCAL also uses 3D PD ultrasound to acquire volumes, but offline assessment takes place in a 2D setting, instead of a 3D setting, let alone a VR environment. Some studies have used VOCAL preconceptionally to assess associations of (sub)endometrial vascularization with pregnancy rates after assisted reproduction[7,8]. In pregnancy, this technique has mainly been used in the late first trimester or thereafter[26,27]. Only Ballering *et al.* have investigated such measurements in the early first trimester in 48 women between 8-12 weeks GA and found that early placental vascular development is different in nulliparous women from multiparous women[28].

The addition of VR when assessing the uterine, placental and embryonic vascularization is unique, because it enables evaluation after ultrasound acquisition and fully benefits the third dimension resulting in more reliable and detailed quantification of vascular volumes. We already have wide experience in using VR for measurement and assessment of uterine, placental, embryonic and fetal structures[15,16,18,26,28].

An advantage of the VR desktop is that it could potentially be integrated within existing ultrasound machines for application in any clinical setting. The system is less expensive and has fewer logistical constraints, using only a regular personal computer with V-Scope software, a 3D monitor or television screen and attached tracking system[17]. These factors facilitate research collaborations and clinical use of the VR desktop.

To date, imaging methods to assess periconceptional utero-placental health remain limited. Using 3D PD ultrasound enables minimally invasive and complete visualization of *in vivo* human utero-placental vasculature. Therefore, we hypothesize that utero-placental vascular volumes can be used as potential markers of endometrial and placental health and pregnancy outcome. Describing the vasculature by different parameters preconceptionally (UVV) and in pregnancy (PVV and EVV) allows to relate separate volumes to individual patient characteristics, such as parity, maternal lifestyle and reproductive complications that affect endometrial quality, placentation, embryonic health and (adverse) pregnancy outcome. Ultimately, 3D PD

ultrasound measurements could be part of a move towards more accurate prevention and treatment strategies starting as early as the periconception period[12,13].

During the periconception period, ultrasound is a safe method to assess uterine, embryonic and placental structures. International guidelines as set for obstetric scanning throughout pregnancy and the ALARA-principle were followed[23-25]. Further, 3D PD ultrasound volume acquisition time was much lower than exposure time during traditional two-dimensional (2D) scanning and the embryo only occupied a small segment in the 3D volume, therefore receiving minimal insonation[29,30].

There are limitations for our study. Firstly, the use of 3D PD image quality relies on ultrasound settings and is sensitive to artefacts. To achieve optimal comparability, ultrasound machine settings were standardized for all patients based on several expert opinions. It appears that the pulse repetition frequency (PRF) is the principal factor influencing 3D PD ultrasound image quality by affecting detection sensitivity for vascular blood flow. It is therefore necessary, that the effects of various settings to optimize acquisition of 3D utero-placental vascular images are evaluated in future studies. Secondly, even if the impact of maternal movements was reduced to a minimum during volume acquisition, factors such as maternal adiposity and artefacts due to embryonic movement or uterine position (ante- or retroverted) can still interfere with image quality. In this study, we have not evaluated effects of characteristics such as maternal BMI or parity on image quality, but in none of the volumes quality was so low that measurements could not be performed. Thus, a larger study population with longitudinal data collection is necessary to establish normal distributions for utero-placental vascularization and associations of these measurements with maternal characteristics, embryonic growth trajectories and pregnancy outcome.

In conclusion, preconceptional and first trimester utero-placental vascular volume measurements using 3D PD ultrasound in the I-Space and VR desktop system are feasible and reliable. These results support the need for future larger cohort studies to improve measurement precision. They also support further investigation of the efficacy of utero-placental vascular measurements by VR as potential markers for uterine and placental function and ultimately their use for prediction and prevention of adverse placenta-related outcomes.

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ADDENDUM

Supplemental Table 1. Measurements and intra- and inter-observer reliability parameters of utero-placental vascular volumes using the Virtual Reality Desktop system

		¤		Intra-observer variability					Inter-observer variability			
		Median **[range] (cm³)**		**ICC**	**Mean difference** **(cm³)** **[+ 95% CI]**		**Relative mean** **difference (%)** **[+ 95% CI]**		**ICC**	**Mean difference (cm³)** **[+ 95% CI]**		**Relative mean difference (%)** **[+ 95% CI]**
Preconception *(N=5)*	**UVV**	1.66	[0.93;5.26]	0.99	0.22	[-0.01;0.84]	4.3	[8.0;14.2]	0.92	-0.40	[-0.70;0.10]	-18.5 [-35.6;-1.4]
Pregnancy 7 weeks GA *(N=10)*	**PVV** EVV	0.40 0.03	[0.08;0.99] [0.01;0.17]	0.99 0.99	0.01 0.00	[-0.02;0.04] [0.00;0.01]	2.7 3.5	[-3.5;8.9] [-5.0;12.0]	0.87 0.63	-0.03 -0.03	[-0.13;0.06] [-0.07;0.01]	21.5 [-7.4;50.4] -36.7 [-86.9;13.4]
9 weeks GA *(N=10)*	**PVV** **EVV**	4.83 0.46	[1.81;9.03] [0.25;0.82]	0.96 0.95	-0.23 0.00	[-0.67;0.21] [-0.04;0.04]	-3.3 -0.1	[-11.8;5.3] [-9.9;9.6]	0.86 0.96	0.02 0.00	[-0.56;0.60] [-0.04;0.03]	-0.6 [-16.1;15.0] -2.4 [-9.6;4.8]
11 weeks GA *(N=10)*	**PVV** **EVV**	8.04 1.45	[2.07;20.58] [0.57;2.16]	0.97 0.99	0.27 -0.03	[-0.33;0.86] [-0.08;0.02]	3.7 -1.6	[-7.9;15.3] [-4.9;1.8]	0.79 0.95	1.29 0.05	[-0.29;2.86] [-0.06;0.15]	21.4 [-7.8;50.5] 3.8 [-3.6;11.3]

ICC = intraclass correlation coefficients; CI = confidence interval; GA = gestational age; UVV = uterine vascular volume; PVV = placental vascular volume; EVV = embryonic vascular volume.

Supplemental Figure 1. Flowchart acquisition of ultrasound volumes and Virtual Reality measurements
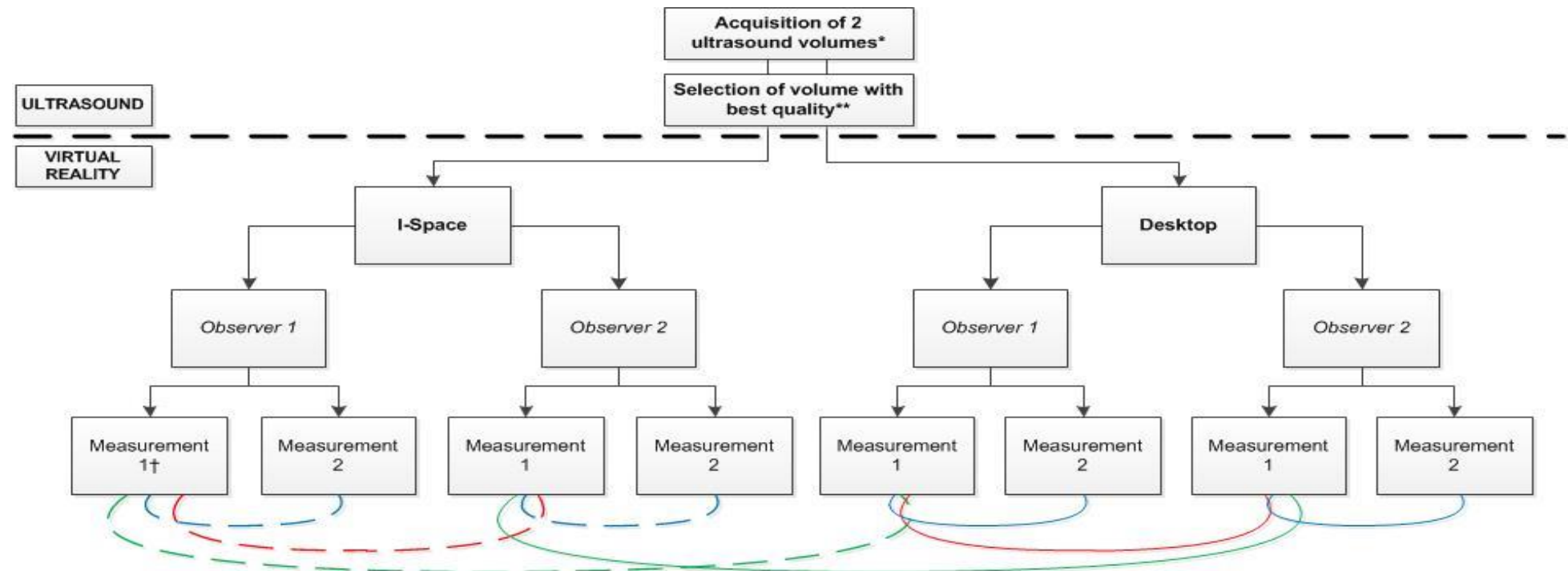
– – – A. Intra-observer agreement (for observer 1 and 2 seperately) using the I-space
– – – B. Inter-observer agreement (between observer 1 and 2) using the I-space
– – – C. Inter-system agreement (I-space vs. VR desktop) of observer 1, measurement 1
––––– D. Intra-observer agreement (for observer 1 and 2 seperately) using the VR desktop
––––– E. Inter-observer agreement (between observer 1 and 2) using the VR desktop
––––– F. Inter-system agreement (I-space vs. VR desktop) of observer 2, measurement 1

* Ultrasound performed preconceptional or in pregnancy (at either 7, 9 or 11 weeks gestational age), acquisition of 2 volumes per patient: first volume in a midsagittal plane, second volume 90 degrees perpendicular to the first plane.

** Items scored for assessment of volume quality: presence of artifacts due to maternal/fetal movements (yes/no), presence of shadows (yes/no), volume completeness (yes/no), placental location in relation to transducer (close/far).

† Measurement of utero-placental vascular volumes: preconceptional uterine vascular volume (UVV, in cm^3); in pregnancy placental vascular volume (PVV, in cm^3) and embryonic vascular volume (EVV, in cm^3).

Supplemental Figure 2. Correlation between utero-placental vascular volumes in two VR systems

a. Preconception: UVV
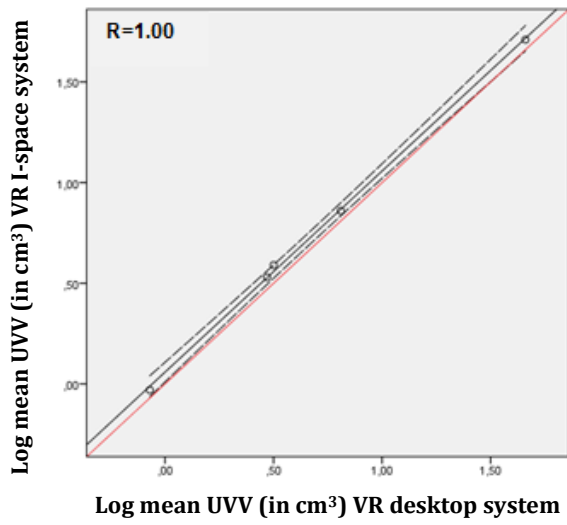
b. Pregnancy (7 weeks GA): PVV
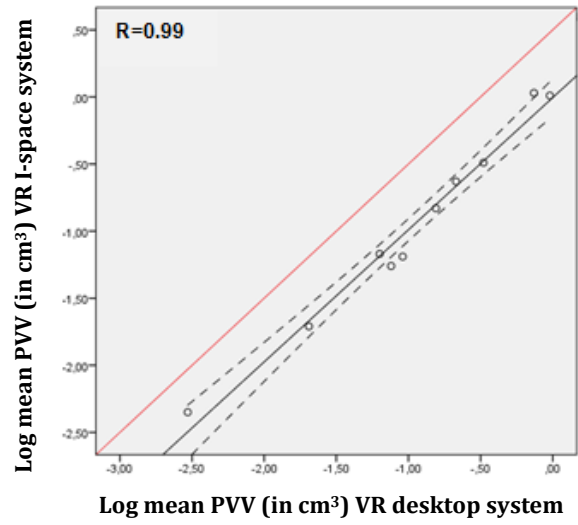
c. Pregnancy (9 weeks GA): PVV
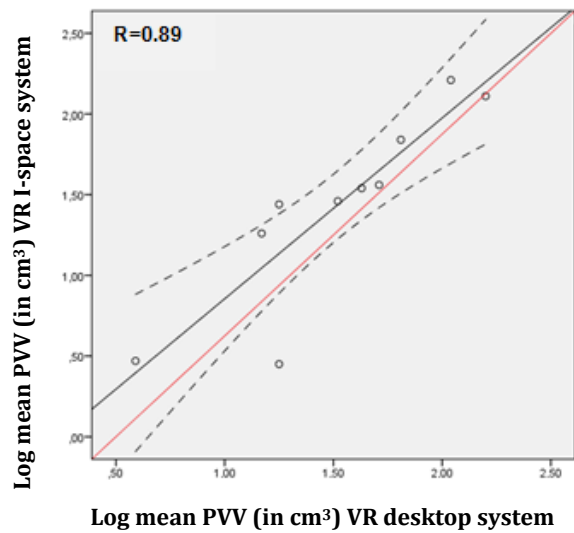
d. Pregnancy (11 weeks GA): PVV
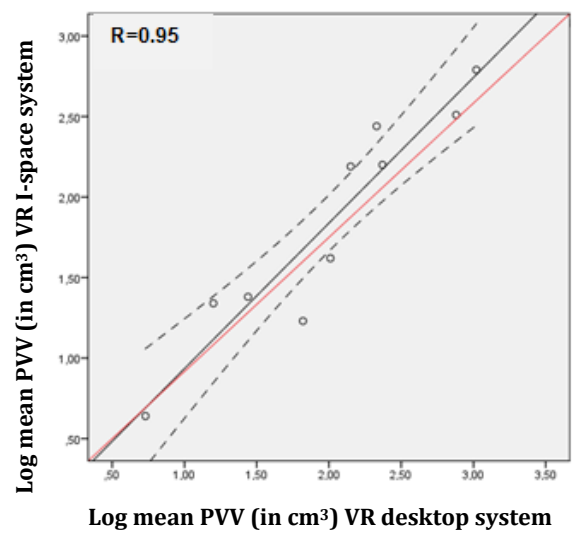
UVV = uterine vascular volume; PVV = placental vascular volume
Log = natural log transformation of UVV and PVV to achieve a normal distribution
R = Pearson's correlation coefficient
———————— *Correlation line*
- - - - - - - - - *95% confidence interval for correlation*
———————— *Reference line (optimal correlation)*

CHAPTER 4

First-trimester utero-placental (vascular) development and embryonic and fetal growth: The Rotterdam Periconception cohort

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ABSTRACT

Introduction: Impaired placental development is a major cause of fetal growth restriction (FGR) and early detection will therefore improve antenatal care and birth outcomes. Here we aim to investigate serial first-trimester ultrasound markers of utero-placental (vascular) development in association with embryonic and fetal growth.

Methods: In a prospective cohort, we periconceptionally included 214 pregnant women. Three-dimensional power Doppler ultrasonography at 7, 9 and 11 weeks gestational age (GA) was used to measure placental volumes (PV) and basal plate surface area by Virtual Organ Computer-aided AnaLysis™, and utero-placental vascular volume (uPVV), crown-rump length (CRL) and embryonic volume (EV) by a V-scope volume rendering application. Estimated fetal weight (EFW) was measured by ultrasound at 22 and 32 weeks GA and birth weight centile (BW) was recorded. Linear mixed models and regression analyses were applied and appropriately adjusted. All analyses were stratified for fetal sex.

Results: PV trajectories were positively associated with CRL (β_{adj}=0.416, 95%CI:0.255;0.576, p<0.001), EV (β_{adj}=0.220, 95%CI:0.058;0.381, p=0.008) and EFW (β_{adj}=0.182, 95%CI:0.012;0.352, p=0.037). uPVV trajectories were positively associated with CRL (β_{adj}=0.203, 95%CI 0.021;0.384, p=0.029). In girls, PV trajectories were positively associated with CRL (p<0.001), EV (p=0.018), EFW (p=0.026), and uPVV trajectories were positively associated with BW (p=0.040). In boys, positive associations were shown between PV trajectories and CRL (p=0.002), and between uPVV trajectories and CRL (p=0.046).

Discussion: First-trimester utero-placental (vascular) development is associated with embryonic and fetal growth, with fetal sex specific modifications. This underlines the opportunity to monitor first-trimester placental development and supports the associations with embryonic and fetal growth.

INTRODUCTION

Worldwide, fetal growth restriction (FGR) is a main problem in perinatal care, because of the high neonatal morbidity and mortality as well as the health sequelae for these children later in life[1-3]. Impaired placental functioning is a major determinant of FGR and is mainly diagnosed in the second half of pregnancy[4]. The most prevalent cause of FGR is malperfusion of the utero-placental circulation resulting from impaired spiral artery remodeling. To meet the crucial maternal vascular adaptation to pregnancy, this process of remodeling already starts in the first trimester of pregnancy[5-7].

The relationship between embryonic and fetal growth is illustrated by the observed associations between first-trimester embryonic growth, mid-pregnancy fetal size, and birth weight[8]. Since FGR can be caused by reduced placental functioning, our hypothesis is that utero-placental (vascular) development in the first trimester of pregnancy impacts embryonic and fetal growth parameters. Moreover, fetal sex dependency is an increasing issue in perinatal medicine, which we assume to be a modifier of these associations[9-11].

To investigate our hypothesis, reliable and non-invasive markers of utero-placental (vascular) development are needed. Ultrasound imaging is mainly used to assess uterine artery blood flow and to quantify placental volume (PV), basal plate surface area and placental vascularization indices using Virtual Organ Computer-aided AnaLysis (VOCAL) in the second half of pregnancy[12-18]. VOCAL uses two-dimensional (2D) planes and the third dimension is not used entirely. Herein lies the advantage of virtual reality (VR) using V-Scope software, enabling actual depth perception to be used for reliable semi-automated offline measurements of first-trimester embryonic growth parameters, i.e., serial crown-rump length (CRL) and embryonic volume (EV) and utero-placental vascular volume (uPVV)[19-22].

In order to contribute to the identification of pregnancies at risk of FGR at the earliest possible moment in pregnancy, we aim to investigate first-trimester serial PV and uPVV measurements, as markers of utero-placental (vascular) development, in association with embryonic and fetal growth in a fetal sex dependent manner.

METHODS

Study Population
The Virtual Placenta study (registration number Dutch Trial Register: NTR6854) is performed as a nested cohort in the Rotterdam Periconception Cohort, an ongoing prospective study conducted at the Department of Obstetrics and Gynecology of the Erasmus MC, University Medical Centre in Rotterdam, the Netherlands[21]. From January 2017 until March 2018, women pregnant less than 10 weeks of gestation were invited to participate. Excluded from analysis were miscarriages, oocyte donations, twins and drop outs. The study protocol was approved by the Erasmus MC Institutional Review Board (MEC 2015-494) on January 20th, 2016. Participating women and partners signed written informed consent at enrolment, also on behalf of their unborn child.

Eligible for inclusion were pregnancies naturally conceived, including intra-uterine insemination (IUI), and after in vitro fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI). Natural pregnancies were dated based on the first day of the last menstrual period (LMP) in regular cycles between >25 and <32 days. Gestational age (GA) was estimated using crown-rump length (CRL) in irregular cycles, unknown LMP, or when GA based on LMP differed more than six days from the GA estimated by CRL. The insemination date was used to calculate GA in IUI pregnancies. In IVF/ICSI pregnancies, GA was calculated from oocyte pick-up day plus 14 days, and, for cryopreserved embryo transfer, from the transfer day plus 19 days.

Study Parameters

Maternal characteristics were obtained from self-reported questionnaires filled out upon enrolment and verified in a personal interview at study entry by a research nurse. Height and weight measurements were standardized to calculate first-trimester body-mass index (BMI). Geographic origin was categorized as Dutch, Western and Non-Western[23]. Educational level was categorized as low, middle or high according to the classification of Statistics Netherlands[24]. To follow up on birth outcomes, mothers filled out a postpartum questionnaire, which were cross-checked with the medical records.

Placenta-related complications were defined as pregnancy-induced hypertension (PIH), preeclampsia (PE), FGR, preterm birth (PTB) and/or small-for-gestational age (SGA). PIH was defined as systolic blood pressure above 140 mmHg or diastolic blood pressure above 90 mmHg after 20 weeks of gestation without signs of hypertension prior to pregnancy or presence of proteinuria[25]. PE was defined as hypertension after 20 weeks of gestation and presence of more than 300 mg proteinuria in a 24 hour period[25,26]. FGR was defined as fetal abdominal circumference and/or estimated fetal weight (EFW) below the 10th centile according to Hadlock curves or a more than twenty centile decrease on the growth curve with a measurement interval of at least two weeks[27,28]. PTB was defined as GA at birth below 37 weeks. Standardized birth weight centiles were calculated from the Perinatal Registration of Newborns in the Netherlands (PRN), established in 2008[29] and SGA was defined as a birth weight below the 10th centile[25,30].

Ultrasound

The participating women underwent 3D transvaginal ultrasound examinations at 7, 9 and 11 weeks GA to obtain volumes encompassing the whole pregnancy, including the embryo, gestational sac and placenta. At 22 and 32 weeks GA transabdominal ultrasonography was performed to estimate fetal growth by assessment of parameters to calculate estimated fetal weight (EFW) using the Hadlock formula, including biparietal diameter, head circumference, abdominal circumference and femur length[31]. Ultrasound examinations were performed by trained sonographers using Voluson E8 or E10 ultrasound systems (GE Medical Systems, Zipf, Austria), using a transvaginal 6-12 MHz transducer in the first trimester and an abdominal rm6c transducer in the second and third trimester. The utero-placental vasculature was visualized using power Doppler ultrasound with standardized settings (power Doppler gain '-8.0', pulse repetition frequency '0.6 kHz', wall motion filter 'low1', quality 'high'). To minimize artifacts and measurement errors caused by movement, participants were asked to hold their

breath for approximately 30 seconds during image acquisition. All (3D) ultrasound examinations were performed according to international guidelines on safe use of Doppler ultrasound in the first trimester of pregnancy and as such, total scanning time was kept as low as possible (ALARA-principle) and always <30 minutes to avoid unnecessary exposure[32,33].

Offline measurements

Using VOCAL™ (4D View, GE Medical System), the trophoblast was traced to measure PV offline[13]. The basal plate surface area (mm^2) was determined by measuring the longest diameter of the placental base plate in a sagittal plane at the level of the utero-placental interface. The length was then traced using electronic calipers. Then the longest diameter was measured in the transverse plane, 90 degrees perpendicular to the sagittal plane. The surface area of the placenta was estimated using the following formula: sagittal length x transverse length x π/4. The placental thickness (mm) was measured underneath the cord insertion. Placental ellipsivity was assessed from the ratio between the largest and the smallest diameter[17,18].

The in-house developed V-scope volume rendering application was used to measure CRL, embryonic volume (EV) and uPVV offline in VR[19,34]. The method for uPVV measurement was applied as previously described with good to excellent intra-observer and inter-observer agreement[20]. Each recording was scored by a self-developed quality score based on presence of artifacts due to maternal and/or embryonic movements (yes/no), presence of acoustic shadowing (yes/no), volume completeness (complete/incomplete), placental position in relation to the transducer (far/close) and overall quality (low/average/good)[20]. The volume with the best score or, in cases of equal scores, the first volume was used for further analysis. Ultrasound datasets with insufficient quality were excluded from measurement and thus analysis.

Using a threshold of the 8-bit (range 0-255) Doppler magnitude data, semi-automatic volume measurements of the utero-placental vasculature were obtained. To enable the most optimal visualization of the utero-placental vasculature the lower-Doppler threshold level was set at a value of 100, meaning that only voxels with a Doppler value of 100 or higher are colored and counted. After removal of embryonic structures, the uPVV was generated by erasing all vascular voxels using a virtual brush up to the utero-placental border which was identified by differences in grey values. The uPVV, as a representation of the maternal utero-placental vascular bed, was then measured using threshold-based segmentation[20]. A ratio was calculated between uPVV and PV to estimate a vascular index of the placenta.

Statistics

Data are presented as median [interquartile range (IQR)] or n (%). Differences in baseline characteristics for conception mode were assessed by chi-square test or Mann-Whitney U test as appropriate. Because of skewed distributions, all volumetric measurements (i.e. PV, uPVV and EV) were transformed using a square root for non-volumetric parameters and a cubic root for volumetric parameters.

Reference values for placental measurements were modelled in a curve (trajectory) using the R Gamlss package (RStudio statistics). To investigate associations between the placental

trajectories and embryonic and fetal growth, a two-step process was followed, taking into account correlations between serial measurements in each pregnancy. First, linear mixed models were used to calculate the subject-specific estimated random effects to extract standardized random intercepts and slopes, summarizing individual trajectories of longitudinal measurements (i.e. PV, uPVV, uPVV/PV ratio, CRL, EV, EFW and birth weight centile), with GA as independent variable. Z-scores were calculated for all continuous data, to compare values for different normal distributions.

In the second stage, z-scores of the estimated random effects from the mixed effects models were used as covariate in linear regression analyses for the trajectories of the first-trimester indices of placental development and the association with embryonic growth and fetal growth. Potential confounders were selected based on the characteristics of the study population and from literature. Associations between placental trajectories and embryonic and fetal growth were assessed in a model adjusted for GA (model 1). The second model (model 2) was additionally adjusted for maternal age, parity, conception mode, BMI and preconception initiation of folic acid supplement use. Next, we performed a stratified analysis for fetal sex (model 1 and 2). All models were constructed based on a combination of both an available placental and embryonic/fetal measurement of sufficient quality, resulting in a varying number of measurements per patient to be used for the analyses. As a final step, all prior analyses were repeated in a subgroup of pregnancies without complications to estimate the impact of placenta-related pregnancy complications on the associations. The required cubic transformation of the data and the calculation of trajectories makes the interpretation of data more complex. Therefore, in order to present the results in the simplest form possible, correction for multiple testing was not applied.

The single measurements of the basal plate surface area, placental thickness and placental ellipsivity were also analysed in the associations with embryonic and fetal growth using linear regression analyses.

All analyses were performed using SPSS software (version 25.0; SPSS Inc., Chicago, IL, USA) and RStudio Statistics (version 3.5.0, 2018) and R (version 3.5.0, R Core team 2018). P-values ≤0.05 were considered statistically significant.

RESULTS

In **Figure 1** the flow chart of the study population is depicted. From a total of 241 pregnancies, 27 pregnancies were excluded for analysis: 22 non-vital pregnancies, 1 withdrawal, and 4 oocyte donations. From the ongoing 214 pregnancies, 466 3D ultrasound datasets were available, of which 328 (70.4%) were usable for measurements of PV and 346 (74.2%) for uPVV. The quality of the datasets was good in 60 (12.9%), average in 215 (46.1%) and low in 80 (17.2%).

Because of the high percentage of IVF pregnancies in our cohort, **Table 1** represents the baseline characteristics of the total study population with stratification for mode of conception. The average maternal age was 32 years and most women were of Dutch geographic origin and intermediate or high educated, folic acid supplement use was up to 98%, 26% used alcoholic drinks and 13% smoked. In contrast to the IVF/ICSI group, women in the naturally conceived

pregnancy group were slightly younger with a higher BMI and reported a lower frequency of preconception initiation of folic acid supplement use.

Figure 1. Flow chart of the study population


```
Enrollment                    Informed consent (N=241)
                                                              No follow-up (N=23):
                                                              ♦ Miscarriage (N=22)
                                                              ♦ Withdrawal (N=1)
First-trimester               Ongoing pregnancies
study visit                   (N=218)
                                                              Exclusion (N=4):
                                                              ♦ Oocyte donation (N=4)

Placental                     Ongoing pregnancies (N=214),
Ultrasonography               Placental datasets (N=466)
                              ♦ 7 weeks GA (N=101)
                              ♦ 9 weeks GA (N=182)
                              ♦ 11 weeks GA (N=183)

Placental
measurements

Placental volume (PV)         Utero-placental vascular        Two-dimensional placental
measurements using            volume (uPVV) measurements      measurements using VOCAL at
VOCAL (N=328)                 using VR (N=346)                11 weeks GA
♦ 7 weeks GA (N=88)           ♦ 7 weeks GA (N=82)             ♦ Placental thickness (N=100)
♦ 9 weeks GA (N=138)          ♦ 9 weeks GA (N=151)            ♦ Placental shape (N=99)
♦ 11 weeks GA (N=102)         ♦ 11 weeks GA (N=113)           ♦ Basal plate surface area
                                                                (N=99)
```

GA = gestational age; VOCAL = Virtual Organ Computer-aided AnaLyzis; VR = virtual reality

In the total study population of 214 pregnancies, 55 women developed placenta-related pregnancy complications (25.7%) comprising of maternal pregnancy complications: 4.2% PIH and 3.3% PE, and of adverse birth outcomes: 7.0% FGR, 8.4% SGA and 10.7% PTB. There were no significant differences in the prevalence of maternal pregnancy complications or adverse fetal outcomes between the naturally conceived and IVF/ICSI pregnancies.

In **Supplemental Figure 1** the first-trimester increase of the trajectories of PV, uPVV, and constant uPVV/PV ratio is depicted for both uncomplicated pregnancies and pregnancies with any placenta-related complication. In **Supplemental Figure 2** the first-trimester trajectories of PV, uPVV and the uPVV/PV ratio are depicted, stratified for fetal sex.

The estimates of these measurements at 7, 9 and 11 weeks are presented in **Supplemental Table 1**.

Table 1. Baseline characteristics of the Virtual Placenta study population

Characteristic	Total group (N=214)	Spontaneous (N=127)	IVF/ICSI (N=87)	*p*-value
Maternal				
Age, years	32.1 [29.0;35.5]†	31.5 [28.9;34.6]†	33.1 [29.3;36.4]†	**0.010***
Nulliparous	115 (53.7%)	68 (53.5%)	47 (54.0%)	0.945
GA at first visit, days	55 [51;65]†	61 [52;65]†	54 [51;64]†	0.095
Geographic origin				0.578
Dutch	165 (77.1%)	95 (74.8%)	71 (81.6%)	
Western other	6 (2.8%)	4 (3.1%)	2 (2.3%)	
Non-western	38 (17.8%)	24 (18.9%)	14 (16.1%)	
Educational level				0.702
Low	18 (8.4%)	10 (7.9%)	8 (9.2%)	
Intermediate	69 (32.2%)	38 (29.9%)	31 (35.6%)	
High	123 (57.5%)	75 (59.1%)	48 (55.2%)	
BMI first trimester, measured (kg/m^2)	24.9 [22.2;28.4]†	25.3 [22.5;29.6]†	24.1 [21.0;26.7]†	0.038*
Folic acid supplement use	210 (98.1%)	123 (96.9%)	87 (100%)	0.095
Preconception initiation	175 (81.8%)	90 (70.9%)	85 (97.7%)	<0.001*
Periconceptional alcohol consumption	57 (26.6%)	34 (26.8%)	23 (26.4%)	0.799
Periconceptional smoking	28 (13.1%)	16 (12.6%)	12 (13.8%)	0.816
Maternal pregnancy complications				
Any placenta-related pregnancy complication**	55 (25.7%)	38 (29.9%)	17 (19.5%)	0.088
PIH	9 (4.2%)	6 (4.7%)	3 (3.4%)	0.648
PE	7 (3.3%)	4 (3.1%)	3 (3.4%)	0.904
Fetal outcomes				
Fetal sex, boys	106 (49.5%)	68 (53.5%)	38 (43.7%)	0.282
GA at birth, days	274 [266;280]†	273 [264;279]†	274 [267;282]†	0.060
Birth weight, grams	3305 [2930;3565]†	3290 [2880;3562]†	3338 [3029;3583]†	0.357
FGR	15 (7.0%)	11 (8.7%)	4 (4.6%)	0.253
SGA	18 (8.4%)	14 (11.0%)	4 (4.6%)	0.096
PTB	23 (10.7%)	16 (12.6%)	7 (8.0%)	0.291
Congenital anomalies	8 (3.7%)	5 (3.9%)	3 (3.4%)	0.853

*Significance at p≤0.05 assessed by chi-square test or Mann Whitney U test as appropriate. **Specified as PIH or PE and/or FGR, PTB and SGA; diagnoses may be overlapping.† Expressed as median [interquartile range, p25-p75]. BMI = body-mass index; FGR = fetal growth restriction; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; PE = preeclampsia; PIH = pregnancy-induced hypertension; PTB = preterm birth; SGA = small-for-gestational age*

Embryonic and growth trajectories

In **Table 2** positive associations are shown between PV trajectories and CRL (model 2: β= 0.416, 95% CI 0.255;0.576, *p*<0.001) and EV (model 2: β= 0.220, 95% CI 0.058;0.381, *p*=0.008). uPVV

trajectories were positively associated with CRL (model 2: β= 0.203, 95% CI 0.021;0.384, *p*=0.029). No significant associations were observed for uPVV/PV ratio trajectories.

A positive association is shown between PV trajectories and EFW (model 2: β= 0.182, 95% CI 0.012;0.352, *p*=0.037).

Stratified analyses for fetal sex

In **Table 3**, in boys positive associations are shown between PV trajectories and CRL (model 2: β= 0.409, 95% CI 0.156;0.661, *p*=0.002) and between uPVV and CRL (model 2: β= 0.252, 95% CI 0.005;0.500, *p*=0.046). In boys, no associations were shown with fetal growth trajectories.

In girls, PV trajectories were positively associated with CRL trajectories (model 2: β= 0.442, 95% CI 0.226;0.658, *p*<0.001) and EV trajectories (model 2: 0.286, 95% CI 0.050;0.522, *p*=0.018) **(Table 3)**. In addition, PV trajectories were positively associated with trajectories of EFW (model 2: β= 0.259, 95% CI 0.032;0.486, *p*=0.026). A positive association was established between uPVV trajectories and birth weight centile (model 2: β= 0.269, 95% CI 0.013;0.525, *p*=0.040).

Sensitivity analysis of uncomplicated pregnancies

All prior analyses were repeated in a subgroup of uncomplicated pregnancies (*n*=159) to estimate the impact of placenta-related complications on the associations.

In **Table 4** positive associations are shown between PV trajectories and CRL (model 2: β= 0.473, 95% CI 0.276;0.670, *p*<0.001) and EV (model 2: β= 0.330, 95% CI 0.117;0.543, *p*=0.003). A negative association was observed between uPVV/PV ratio trajectories and fetal growth estimated by EFW trajectories (model 2: β= -0.338, 95% CI -0.675;-0.001, *p*=0.049).

When stratified for fetal sex, uncomplicated pregnancies demonstrated positive associations in boys between PV trajectories and CRL (model 2: β= 0.346, 95% CI 0.023;0.669, *p*=0.037) **(Table 5).** In girls, uncomplicated pregnancies demonstrated positive associations of PV trajectories with CRL trajectories (model 2: β= 0.662, 95% CI 0.407;0.916, *p*<0.001) and EV trajectories (model 2: β= 0.562, 95% CI 0.245;0.879, *p*=0.001). Also in girls, a positive association was established between uPVV trajectories and birth weight centile (model 2: β= 0.156, 95% CI 0.003;0.309, *p*=0.046).

Additional analysis of placental thickness, placental ellipsivity and basal plate surface area

In **Supplemental Table 2** a positive association is shown between placental thickness and PV at 11 weeks GA (model 2: β= 0.351, 95% CI 0.165;0.537, p<0.001) and the basal plate surface area (model 2: β= 0.329, 95% CI 0.131;0.526, p<0.001). A negative association was demonstrated between placental ellipsivity and EFW trajectories in girls only (model 2: β= -0.278, 95% CI -0.554;-0.001, p=0.049) **(Supplemental Table 4)**. No significant associations were observed for basal plate surface area.

Table 2. Associations between first-trimester trajectories of utero-placental (vascular) development and embryonic and fetal growth

	CRL trajectory (N=154†)				EV trajectory (N=154†)				EFW trajectory (N=136†)				Birth weight centile (p) (N=151†)			
	Model 1 Estimate (β), (95% CI)	*p*-value	**Model 2** Estimate (β), (95% CI)	*p*-value	**Model 1** Estimate (β), (95% CI)	*p*-value	**Model 2** Estimate (β), (95% CI)	*p*-value	**Model 1** Estimate (β), (95% CI)	*p*-value	**Model 2** Estimate (β), (95% CI)	*p*-value	**Model 1** Estimate (β), (95% CI)	*p*-value	**Model 2** Estimate (β), (95% CI)	*p*-value
PV	0.412 (0.253; 0.570)	<0.001**	0.416 (0.255; 0.576)	<0.001**	0.211 (0.051; 0.371)	0.010**	0.220 (0.058; 0.381)	0.008**	0.167 (-0.003; 0.337)	0.054	0.182 (0.012; 0.352)	0.037*	0.079 (-0.017; 0.331)	0.311	0.087 (-0.067; 0.240)	0.265
uPVV	0.218 (0.048; 0.388)	0.012*	0.203 (0.021; 0.384)	0.029*	0.022 (-0.143; 0.187)	0.794	0.008 (-0.167; 0.183)	0.930	-0.012 (-0.194; 0.170)	0.895	-0.056 (-0.244; 0.132)	0.559	0.100 (-0.053; 0.253)	0.200	0.144 (-0.015; 0.303)	0.076
uPVV/ PV ratio	-0.142 (-0.417; 0.133)	0.995	-0.165 (-0.447; 0.118)	0.251	-0.185 (-0.446; 0.075)	0.162	-0.186 (-0.453; 0.081)	0.170	-0.193 (-0.472; 0.087)	0.176	-0.226 (-0.508; 0.056)	0.115	0.114 (-0.131; 0.360)	0.359	0.112 (-0.138; 0.362)	0.379

Model 1: Adjusted analysis for gestational age.

Model 2: Model 1 + adjusted for maternal age, parity, conception mode, body-mass index and preconception initiation of folic acid supplement use.

*† Number of available measurements for analysis of the placenta and embryo/fetus combined. *Significance at p≤0.05; **Significance at p≤0.01.*

CRL = crown-rump length (mm); EFW = estimated fetal weight (gram); EV = embryonic volume (cm³); PV = placental volume (cm³); uPVV = utero-placental vascular volume (cm³).

Table 3. Stratified analysis for fetal sex: associations between first-trimester trajectories of utero-placental (vascular) development and embryonic and fetal growth

	CRL trajectory (N=154†)				EV trajectory (N=154†)				EFW trajectory (N=136†)				Birth weight centile (p) (N=151†)			
	Model 1		**Model 2**		**Model 1**		**Model 2**		**Model 1**		**Model 2**		**Model 1**		**Model 2**	
	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value
Boys (N=72†)																
PV	0.384 (0.136; 0.632)	0.003**	0.409 (0.156; 0.661)	0.002**	0.122 (-0.099; 0.343)	0.273	0.147 (-0.073; 0.367)	0.187	0.012 (-0.266; 0.291)	0.930	0.002 (-0.267; 0.272)	0.985	0.190 (-0.042; 0.422)	0.106	0.193 (-0.040; 0.425)	0.103
uPVV	0.214 (-0.008; 0.437)	0.059	0.252 (0.005; 0.500)	0.046*	-0.005 (-0.198; 0.187)	0.956	0.047 (-0.162; 0.255)	0.657	0.055 (-0.194; 0.303)	0.662	0.040 (-0.217; 0.297)	0.758	0.065 (-0.150; 0.280)	0.549	0.063 (-0.156; 0.281)	0.568
uPVV/ PV ratio	-0.074 (-0.484; 0.337)	0.772	-0.076 (-0.504; 0.351)	0.723	-0.193 (-0.537; 0.150)	0.265	-0.160 (-0.508; 0.188)	0.265	-0.072 (-0.487 ;0.344)	0.731	-0.053 (-0.464; 0.359)	0.799	0.067 (-0.273; 0.407)	0.695	0.121 (-0.227; 0.469)	0.491
Girls (N=80†)																
PV	0.430 (0.216; 0.644)	<0.001**	0.442 (0.226; 0.658)	<0.001**	0.260 (0.026; 0.494)	0.030*	0.286 (0.050; 0.522)	0.018*	0.254 (0.035; 0.472)	0.024*	0.259 (0.032; 0.486)	0.026*	0.011 (-0.199; 0.220)	0.920	-0.010 (-0.222; 0.202)	0.927
uPVV	0.211 (-0.066; 0.487)	0.133	0.163 (-0.127; 0.452)	0.266	-0.024 (-0.274; 0.227)	0.824	-0.014 (-0.313; 0.285)	0.924	-0.051 (-0.329 ;0.226)	0.713	-0.107 (-0.394; 0.181)	0.460	0.259 (0.011; 0.507)	0.041*	0.269 (0.013; 0.525)	0.040*
uPVV/ PV ratio	-0.239 (-0.627; 0.150)	0.323	-0.245 (-0.644; 0.154)	0.225	-0.224 (-0.622; 0.174)	0.266	-0.222 (-0.631; 0.187)	0.283	-0.272 (-0.668; 0.123)	0.174	-0.290 (-0.700; 0.120)	0.163	0.213 (-0.144; 0.571)	0.239	0.167 (-0.203; 0.536)	0.371

Model 1: Adjusted analysis for gestational age.

Model 2: Model 1 + adjusted for maternal age, parity, conception mode, body-mass index and preconception initiation of folic acid supplement use.

*† Number of available measurements for analysis of the placenta and embryo/fetus combined. *Significance at p≤0.05; **Significance at p≤0.01. CRL = crown-rump length (mm);*

EFW = estimated fetal weight (gram); EV = embryonic volume (cm³); PV = placental volume (cm³); uPVV = utero-placental vascular volume (cm³).

Table 4. Associations between first-trimester trajectories of utero-placental (vascular) development and embryonic and fetal growth in uncomplicated pregnancies

	CRL trajectory (N=107†)				EV trajectory (N=107†)				EFW trajectory (N=104†)				Birth weight centile (p) (N=112†)			
	Model 1 Estimate (β), (95% CI)	*p*-value	**Model 2** Estimate (β), (95% CI)	*p*-value	**Model 1** Estimate (β), (95% CI)	*p*-value	**Model 2** Estimate (β), (95% CI)	*p*-value	**Model 1** Estimate (β), (95% CI)	*p*-value	**Model 2** Estimate (β), (95% CI)	*p*-value	**Model 1** Estimate (β), (95% CI)	*p*-value	**Model 2** Estimate (β), (95% CI)	*p*-value
PV	0.467 (0.271; 0.663)	<0.001**	0.473 (0.276; 0.670)	<0.001**	0.318 (0.106; 0.529)	0.004**	0.330 (0.117; 0.543)	0.003**	0.147 (-0.066; 0.361)	0.174	0.168 (-0.043; 0.380)	0.117	-0.010 (-0.120; 0.101)	0.865	-0.004 (-0.115; 0.107)	0.943
uPVV	0.192 (-0.020; 0.404)	0.075	0.150 (-0.079; 0.378)	0.198	0.037 (-0.182; 0.256)	0.738	0.001 (-0.235; 0.236)	0.994	-0.084 (-0.289; 0.122)	0.421	-0.145 (-0.360; 0.070)	0.185	0.001 (-0.104; 0.103)	0.996	0.036 (-0.072; 0.145)	0.508
uPVV/ PV ratio	-0.215 (-0.569; 0.118)	0.230	-0.254 (-0.617; 0.110)	0.169	-0.279 (-0.638; 0.079)	0.126	-0.288 (-0.658; 0.082)	0.126	-0.303 (-0.635; 0.030)	0.074	-0.338 (-0.675;-0.001)	0.049*	-0.039 (-0.209; 0.130)	0.646	-0.004 (-0.179; 0.171)	0.965

Model 1: Adjusted analysis for gestational age.

Model 2: Model 1 + adjusted for maternal age, parity, conception mode, body-mass index and preconception initiation of folic acid supplement use.

*† Number of available measurements for analysis of the placenta and embryo/fetus combined. *Significance at p≤0.05; **Significance at p≤0.01.*

CRL = crown-rump length (mm); EFW = estimated fetal weight (gram); EV = embryonic volume (cm³); PV = placental volume (cm³); uPVV = utero-placental vascular volume (cm³).

Table 5. Stratified analysis for fetal sex: associations between first-trimester trajectories of utero-placental (vascular) development and embryonic and fetal growth in uncomplicated pregnancies

	CRL trajectory (N=154†)				EV trajectory (N=154†)				EFW trajectory (N=136†)				Birth weight centile (p) (N=151†)			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	Estimate (β), (95% CI)	*p*-value	Estimate (β), (95% CI)	*p*-value	Estimate (β), (95% CI)	*p*-value	Estimate (β), (95% CI)	*p*-value	Estimate (β), (95% CI)	*p*-value	Estimate (β), (95% CI)	*p*-value	Estimate (β), (95% CI)	*p*-value	Estimate (β), (95% CI)	*p*-value
Boys (N=72†)																
PV	0.288 (-0.022; 0.598)	0.068	0.346 (0.023; 0.669)	0.037*	0.105 (-0.180; 0.390)	0.462	0.143 (-0.148; 0.435)	0.327	-0.006 (-0.330; 0.318)	0.970	-0.023 (-0.342; 0.295)	0.883	0.076 (-0.099; 0.250)	0.387	0.037 (-0.139; 0.213)	0.673
uPVV	0.172 (-0.117; 0.462)	0.238	0.278 (-0.042; 0.598)	0.087	0.002 (-0.259; 0.264)	0.985	0.109 (-0.175; 0.394)	0.443	0.003 (-0.282; 0.287)	0.986	0.062 (-0.248; 0.373)	0.687	-0.120 (-0.268; 0.027)	0.108	-0.113 (-0.276; 0.050)	0.170
uPVV/ PV ratio	-0.185 (-0.690; 0.320)	0.465	-0.055 (-0.613; 0.504)	0.723	-0.220 (-0.667; 0.227)	0.326	-0.059 (-0.541; 0.423)	0.807	-0.171 (-0.643; 0.301)	0.470	0.013 (-0.498; 0.524)	0.959	-0.226 (-0.468; 0.016)	0.066	-0.203 (-0.450; 0.044)	0.104
Girls (N=80†)																
PV	0.608 (0.348; 0.869)	<0.001**	0.662 (0.407; 0.916)	<0.001**	0.472 (0.157; 0.787)	0.004**	0.562 (0.245; 0.879)	0.001**	0.238 (-0.052; 0.528)	0.106	0.249 (-0.053; 0.551)	0.104	-0.074 (-0.217; 0.068)	0.303	-0.092 (-0.237; 0.053)	0.209
uPVV	0.216 (-0.119; 0.550)	0.202	0.099 (-0.255; 0.453)	0.576	0.047 (-0.327; 0.422)	0.801	-0.061 (-0.458; 0.337)	0.761	-0.130 (-0.440; 0.181)	0.405	-0.228 (-0.549; 0.093)	0.160	0.128 (-0.020; 0.275)	0.089	0.156 (0.003; 0.309)	0.046*
uPVV/ PV ratio	-0.281 (-0.808; 0.245)	0.289	-0.325 (-0.862; 0.211)	0.229	-0.393 (-0.971; 0.186)	0.179	-0.437 (-1.041; 0.167)	0.152	-0.386 (-0.873; 0.101)	0.226	-0.429 (-0.937; 0.080)	0.096	0.151 (-0.076; 0.378)	0.188	0.154 (-0.084; 0.391)	0.199

Model 1: Adjusted analysis for gestational age.

Model 2: Model 1 + adjusted for maternal age, parity, conception mode, body-mass index and preconception initiation of folic acid supplement use.

*† Number of available measurements for analysis of the placenta and embryo/fetus combined. *Significance at p≤0.05; **Significance at p≤0.01. CRL = crown-rump length (mm); EFW = estimated fetal weight (gram); EV = embryonic volume (cm³); PV = placental volume (cm³); uPVV = utero-placental vascular volume (cm³).*

DISCUSSION

This study shows that first-trimester PV and uPVV trajectories are associated with embryonic and fetal growth, with some modification by fetal sex. PV trajectories were positively associated with embryonic growth, which was most pronounced in girls. In the same way, uPVV trajectories were positively associated with embryonic growth, in particular in boys and with birth weight centile only in girls.

The positive associations between first-trimester utero-placental (vascular) development and embryonic and fetal growth suggest that serial PV and uPVV can be used as ultrasound markers for the monitoring of early placental development. The stronger effect estimates observed in the subgroup of pregnancies without a placenta-related complication further support that the markers estimate the physiology of placenta-dependent antenatal growth. Placental functioning is also determined by maternal cardiovascular adaptation to pregnancy, trophoblast tissue metabolism, endocrine and immunological pathways, and epigenetics[35]. However, an increase in first-trimester utero-placental (vascular) development does not guarantee better placental functioning and fetal and birth outcome, because a gold standard of optimal utero-placental (vascular) development is lacking. Therefore, further studies should also include personalized characteristics of first trimester placental growth curves in normal and high risk pregnancies.

Our results, however, are in line with known associations between first-trimester placental volume measurements and both placental weight and birth weight[36,37]. In line with previous publications, we demonstrated an association between placental thickness and basal plate surface area at 11 weeks GA and PV at 11 weeks GA[17,18]. Less clear were the associations between placental thickness and basal plate surface area and trajectories of embryonic and fetal growth. A possible explanation is the relatively small number of available measurements at 11 weeks GA and that a 2D ultrasound approach is compared with 3D ultrasound trajectories. The 2D ultrasound parameters might reflect placental development less reliably since this is based on limited ultrasound data (i.e. information provided by 2 planes only). Moreover, we demonstrated that pregnancies complicated by FGR and PE are associated with decreased placental vascularization indices, higher impedance to uterine blood flow and lower serum placental growth factor levels in the second half of pregnancy[14,15]. This could be due to impaired placental vascular development, i.e. deficient spiral artery remodeling. Because these (patho)physiological processes start already in the first trimester of pregnancy, it is very likely that women with a small placenta in early pregnancy are at risk for developing FGR, PE or other placenta-related pregnancy complications[38-40]. Although our study was not aimed and powered to investigate associations between trajectories of PV and uPVV and placenta-related pregnancy complications, the sensitivity analysis showed that the observed associations were not primarily due to these adverse outcomes.

We observed that first-trimester placental development was most strongly associated with first-trimester embryonic development. The association with second- and third-trimester fetal development as well as birth outcomes was weaker. Explanations are that there is indeed a stronger developmental correlation in the first trimester. Moreover, the time frame between 7 and 11 weeks GA covers a period of exceptionally rapid placental and embryonic development. The onset of the feto-maternal circulation after spiral artery unplugging lies in this period,

although the exact timing is known to be variable. As such, placental vascular development may not be as strongly associated as overall placental development, reflected by the association between PV and embryonic growth that was demonstrated from our data. In addition, the first-trimester parameters are temporally remote from the parameters that were assessed from the second trimester onwards. This provides an opportunity for other impacting factors to interact with the later stages of fetal growth. Finally, it needs to be considered that EFW may not be the most robust marker, as the inherent measurement error of EFW by ultrasound could have contributed to the lack of association.

PV trajectories were associated with both increased embryonic and fetal growth, most pronounced in girls. While the association between uPVV trajectories and increased embryonic growth was only present in boys, these trajectories in girls were also associated with a higher birth weight centile. These observed gender modifications in early placental development are in line with previous findings describing that early fetal growth is modified in a fetal sex dependent manner and persists up until birth[9,41]. In addition, it has been shown that girls use more energy for placenta development compared to boys, while boys direct more energy towards body growth and development[42]. The proposed mechanism by which this is regulated suggests an intensive interplay between mother, fetus and placenta[43]. A review on fetal sex dependency and placental growth and function in animal models described that utero-placental trophoblast function in female fetuses was most sensitive to disruptions in the periconception period, while placental trophoblast function in male fetuses was more sensitive to disruptions mid to late gestation[44]. The sensitivity analysis of the subgroup of uncomplicated pregnancies showed, after stratification for fetal sex, stronger positive associations between placenta development, in particular serial PV, and embryonic and fetal growth in girls. Our results substantiate that the sex specific differences in early utero-placental (vascular) development with an impact on embryonic and fetal growth can be due to sex-specific epigenetic programming of in particular imprinted genes, such as IGF_2[45] of which the involved fetal sex specific pathways needs to be unraveled.

The main strengths of our prospective study are its high internal validity due to the recruitment of patients from a single hospital together with the standardized collection of serial 3D ultrasonography and VR measurements from the early first- trimester onwards, precise PV, uPVV, CRL and EV measurements, and the detailed information on baseline characteristics and pregnancy outcome data[21]. We applied advanced statistical models for adjustment of maternal age, parity, conception mode, BMI, and folic acid supplement use, and stratified the analysis for fetal sex. Inherent to the observational study design, we also encountered some limitations. A limitation of our study is the selective participation of high-risk and relatively high-educated Dutch women and a large group of IVF/ICSI pregnancies, which confines external validity. Therefore, we adjusted our analyses for conception mode and recommend validation of our findings in a general population. However, the effects of residual confounding cannot be excluded due to the observational character of this study. Moreover, other factors involved in utero-placental (vascular) development were not evaluated, for example biomarkers derived from placental endocrine and metabolic processes, such as placental growth factor[32].

So far, the availability of the VR technique is not yet widespread. Because of the accuracy and precision of the measurements possibilities for implementation in clinical practice have

emerged[46]. The desktop setting in particular makes VR more easily applicable in a clinical setting. Although this is a one centre study, we have developed broad expertise in our clinic with a broad range of embryonic, fetal and placental measurements in the different trimesters of pregnancy using the VR desktop system[19]. A large number of observers have shown good to excellent intra- and interobserver reproducibility performing VR measurements. Currently, a clinical trial is conducted addressing the detection of congenital anomalies in the first trimester using the VR desktop system. This trial also studies patient and clinician perspectives and satisfaction[47].

It was not our aim to investigate FGR or SGA as a separate group and therefore our study was not powered for this aim. However, in the future it would be most interesting to investigate a large group of strictly defined FGR and SGA based on more robust criteria such as abdominal circumference <3rd centile or abnormal Dopplers. This would distinguish the true FGR from the constitutionally SGA.

The inclusion of PTB in the group of placenta-related complications could be considered as a limitation of this study, because also the pathophysiology of PTB is multifactorial and cannot be solely attributed to suboptimal placental development. In future studies with a larger sample size it would be interesting to investigate PTB as a separate group and stratify the analysis for a placental or non-placental origin.

Our data further support that first-trimester utero-placental (vascular) development is not uniform in every pregnancy and woman, and in the same manner associated with embryonic and fetal growth. Therefore, the next step should be, after confirmation of our findings in the general population, to investigate the predictive value of FGR using first-trimester PV and uPVV measured by 3D ultrasonography and VR. In the meantime, the following topics should be addressed in future research settings. First, calculating PV in VR by semi-automated utero-placental border detection is impossible so far and manual delineation is too time-consuming. Furthermore, despite the used preset, power Doppler ultrasound remains sensitive to artefacts during image acquisition and the selected gain may not be appropriate for all patients in the cohort. For example, obesity may attenuate image quality. Some studies recommend using individualized sub-noise gain to guarantee acquisition with minimum noise artifact[48]. Future exchange of protocols for 3D power Doppler ultrasound settings to measure the utero-placental vasculature could ensure more general recommendations.

Finally, the volume and quality of the uterine vasculature prior to pregnancy and its postconceptional increase could be a determinant of placental development and subsequent fetal growth. Endometrial receptivity enables embryonic implantation, initiating subsequent physiological vascular transformation of spiral arteries into low resistance vessels[49,50]. Endometrial pregnancy preparation and the possible impact of maternal lifestyle exposure on periconceptional endometrial quality, ideally studied in low-risk settings is therefore another interesting topic for future research.

To conclude, first-trimester PV and uPVV trajectories are associated with embryonic and fetal growth, in a fetal sex dependent manner. These findings underline the opportunity to monitor placental development as early as in the first trimester and therefore support the associations with embryonic and fetal growth.

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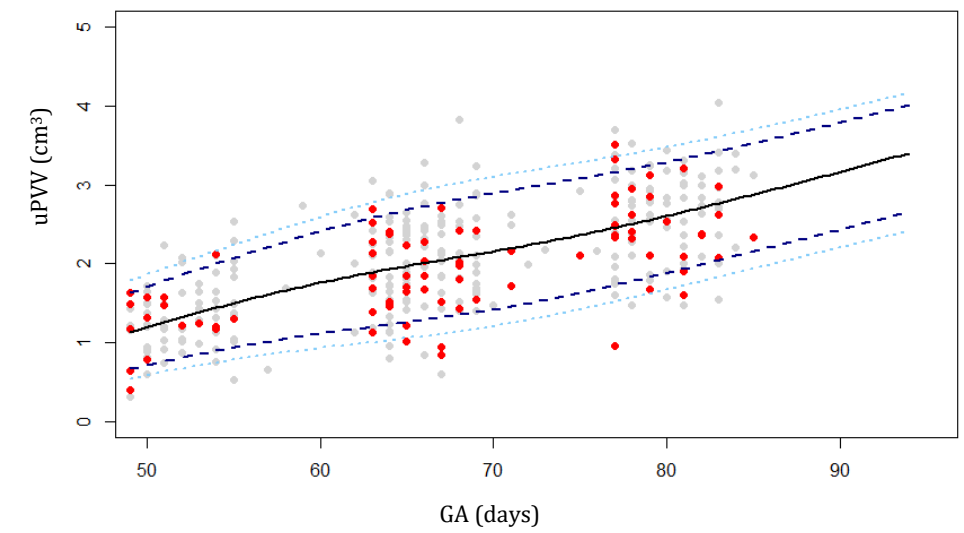
ADDENDUM

Supplemental Figure 1. First-trimester trajectories of utero-placental (vascular) development of the study population (*n*=214)
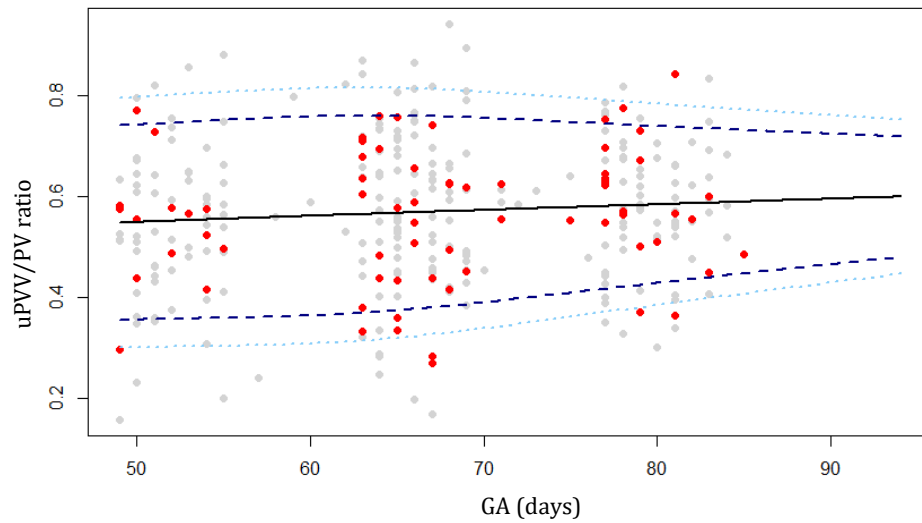
a. PV*
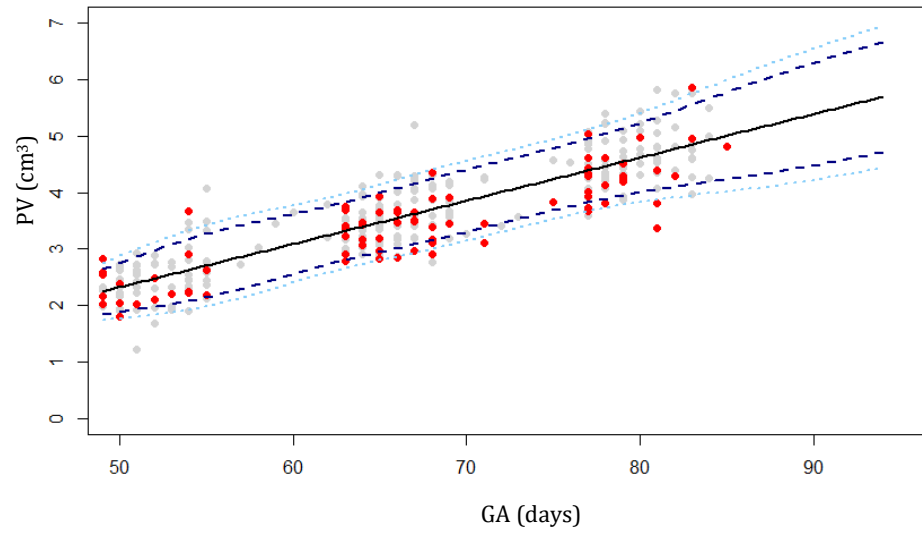
b. uPVV*

c. uPVV/PV ratio*

Lower line (light blue) .... *5th centile (p5)*
Lower line (dark blue) – – – *10th centile (p10)*
Mid line (black) —— *50th centile (p50)*
Upper line (dark blue) – – – *90th centile (p90)*
Upper line (light blue) .... *95th centile (p95)*

Grey dots: uncomplicated pregnancies
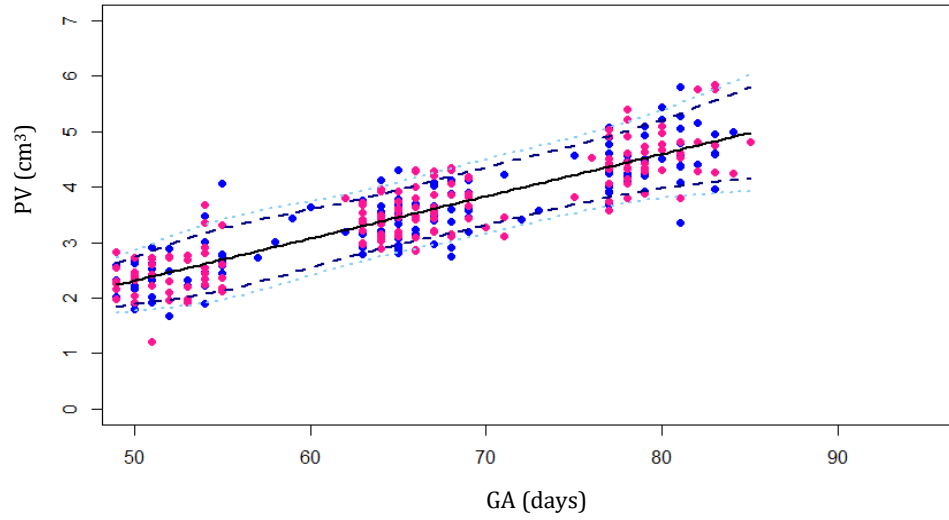*Red dots: pregnancies with any placenta-related complication*
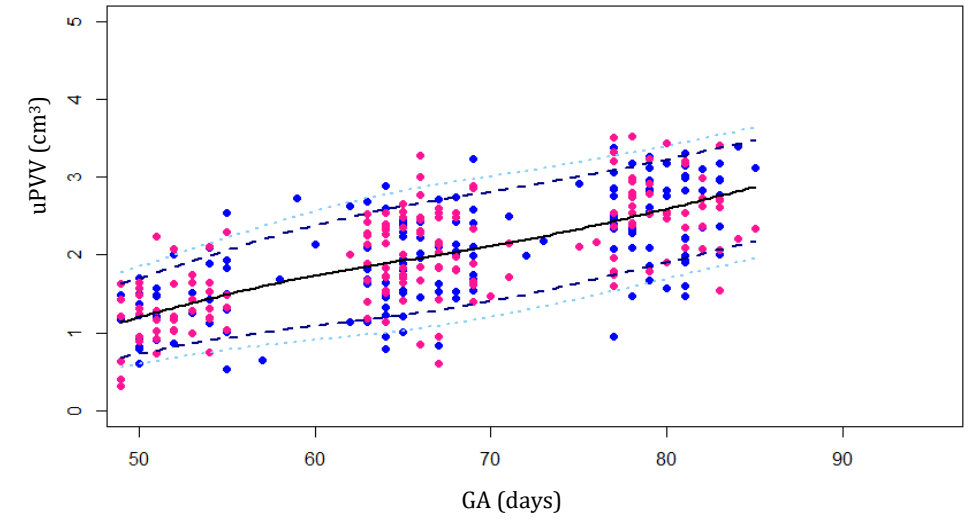
* cubic root transformation

GA = gestational age (days); PV = placental volume; uPVV = utero-placental vascular volume

Supplemental Figure 2. First-trimester trajectories of utero-placental (vascular) development of the study population (n=214), stratified for fetal sex

a. PV*

b. uPVV*

c. uPVV/PV ratio*
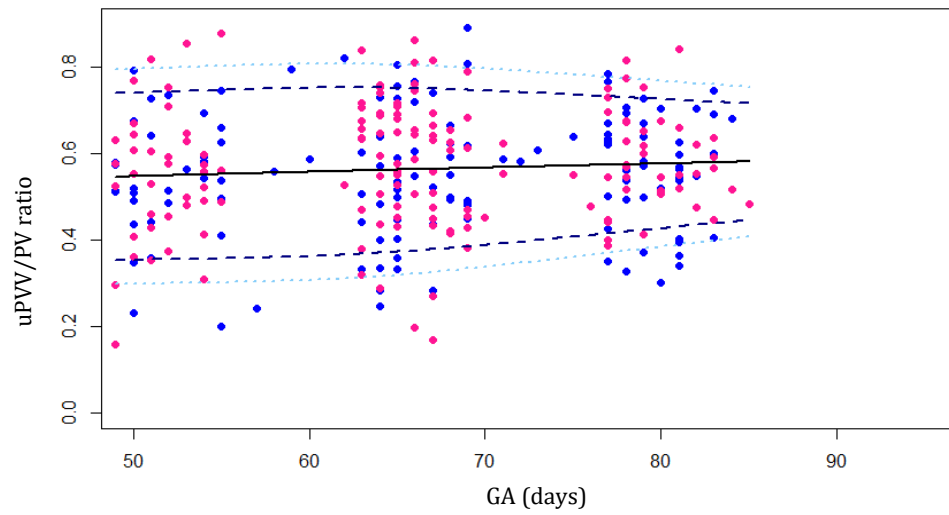
Lower line (light blue) .... *5th centile (p5)*
Lower line (dark blue) – – – *10th centile (p10)*
Mid line (black) —— *50th centile (p50)*
Upper line (dark blue) – – – *90th centile (p90)*
Upper line (light blue) .... *95th centile (p95)*

Blue dots: boys
Pink dots: girls

* cubic root transformation

GA = gestational age (days); PV = placental volume; uPVV = utero-placental vascular volume

Supplemental Table 1. Medians and ranges of measurements of utero-placental (vascular) development per week gestational age (GA) of the study population (n=214)

	7 weeks GA N=103*		9 weeks GA *N*=172*		11 weeks GA N=131*	
	Median	IQR	Median	IQR	Median	IQR
PV (cm³)	14.79	11.40;18.88	47.36	33.07;60.10	102.58	80.55;120.81
uPVV (cm³)	2.21	1.06;4.32	9.26	5.17;15.30	16.34	12.06;32.13
uPVV/PV ratio	0.17	0.10;0.23	0.21	0.12;0.29	0.18	0.12;0.30

Maximum number of usable volumetric datasets per week gestational age

GA = gestational age; PV = placental volume; uPVV = utero-placental vascular volume ; uPVV/PV ratio = ratio between uPVV and PV.

Supplemental Table 2. Associations between placental thickness, placental ellipsivity and basal plate surface area at 11 weeks gestational age (GA) and utero-placental (vascular) development at 11 weeks GA of the study population (n=214)

	PV (N=98†)				uPVV (N=89†)				uPVV/PV ratio (N=88†)			
	Model 1		**Model 2**		**Model 1**		**Model 2**		**Model 1**		**Model 2**	
	Estimate (β), (95% CI)	*p*-value	Estimate (β), (95% CI)	*p*-value	Estimate (β), (95% CI)	*p*-value	Estimate (β), (95% CI)	*p*-value	Estimate (β), (95% CI)	*p*-value	Estimate (β), (95% CI)	*p*-value
PT	0.328 (0.142; 0.513)	0.001**	0.351 (0.165; 0.537)	<0.001**	0.167 (-0.068; 0.402)	0.161	0.157 (-0.085; 0.398)	0.202	-0.106 (-0.331; 0.120)	0.354	-0.133 (-0.361; 0.095)	0.250
Shape	0.143 (-0.059; 0.345)	0.163	0.116 (-0.087; 0.320)	0.259	-0.050 (-0.291; 0.191)	0.679	-0.020 (-0.274; 0.234)	0.874	-0.078 (-0.305; 0.150)	0.499	-0.039 (-0.275; 0.197)	0.742
Basal plate	0.341 (0.148; 0.534)	0.001**	0.329 (0.131; 0.526)	0.001**	0.093 (-0.152; 0.338)	0.452	0.146 (-0.112; 0.404)	0.264	-0.162 (-0.392; 0.069)	0.166	-0.135 (-0.375; 0.104)	0.265

Model 1: Adjusted analysis for gestational age.

Model 2: Model 1 + adjusted for maternal age, parity, conception mode, body-mass index and preconception initiation of folic acid supplement use.

*† Number of available measurements for analysis of the placenta combined. *Significance at p≤0.05; **Significance at p≤0.01.*

Basal plate = basal plate surface area (mm²); PT = placental thickness (mm); PV = placental volume (cm³); Shape = placental ellipsivity; uPVV = utero-placental vascular volume (cm³).

Supplemental Table 3. Associations between placental thickness, placental ellipsivity and basal plate surface area at 11 weeks gestational age (GA) and embryonic and fetal growth of the study population (n=214)

	CRL trajectory (N=86†)				EV trajectory (N=86†)				EFW trajectory (N=83†)				Birth weight centile (p) (N=97†)			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value	Estimate (β), (95% CI)	p-value
PT	0.134 (-0.093; 0.361)	0.243	0.136 (-0.101; 0.372)	0.257	0.104 (-0.112; 0.320)	0.340	0.086 (-0.139; 0.310)	0.450	0.065 (-0.167; 0.297)	0.577	0.027 (-0.211; 0.265)	0.822	0.145 (-0.045; 0.334)	0.311	0.168 (-0.027; 0.363)	0.091
Shape	0.085 (-0.141; 0.312)	0.455	0.075 (-0.160; 0.309)	0.528	0.029 (-0.187; 0.244)	0.792	0.022 (-0.200; 0.244)	0.842	-0.117 (-0.357; 0.123)	0.336	-0.093 (-0.340; 0.153)	0.453	-0.071 (-0.262; 0.121)	0.468	-0.063 (-0.263; 0.137)	0.533
Basal plate	-0.040 (-0.271; 0.191)	0.731	-0.071 (-0.309; 0.168)	0.556	0.068 (-0.151; 0.286)	0.541	0.038 (-0.188; 0.263)	0.741	0.023 (-0.201; 0.247)	0.837	0.030 (-0.201; 0.262)	0.796	0.068 (-0.123; 0.259)	0.480	0.108 (-0.088; 0.304)	0.277

Model 1: Adjusted analysis for gestational age.

Model 2: Model 1 + adjusted for maternal age, parity, conception mode, body-mass index and preconception initiation of folic acid supplement use.

*† Number of available measurements for analysis of the placenta and embryo/fetus combined. *Significance at p≤0.05; **Significance at p≤0.01.*

Basal plate = basal plate surface area (mm²); CRL = crown-rump length (mm); EFW = estimated fetal weight (gram); EV = embryonic volume (cm³); PT = placental thickness (mm); Shape = placental ellipsivity.

Supplemental Table 4. Stratified analysis for fetal sex: associations between placental thickness, placental ellipsivity and basal plate surface area at 11 weeks gestational age (GA) and embryonic and fetal growth of the study population (n=214)

	CRL trajectory (N=86†)				EV trajectory (N=86†)				EFW trajectory (N=83†)				Birth weight centile (p) (N=97†)			
	Model 1 Estimate (β), (95% CI)	p-value	Model 2 Estimate (β), (95% CI)	p-value	Model 1 Estimate (β), (95% CI)	p-value	Model 2 Estimate (β), (95% CI)	p-value	Model 1 Estimate (β), (95% CI)	p-value	Model 2 Estimate (β), (95% CI)	p-value	Model 1 Estimate (β), (95% CI)	p-value	Model 2 Estimate (β), (95% CI)	p-value
Boys (N=72†)																
PT	0.244 (-0.115; 0.604)	0.176	0.247 (-0.166; 0.660)	0.233	0.088 (-0.227; 0.402)	0.576	0.000 (-0.353; 0.352)	0.998	0.265 (-0.081; 0.612)	0.129	0.135 (-0.244; 0.513)	0.473	0.145 (-0.045; 0.335)	0.132	0.164 (-0.040; 0.367)	0.113
Shape	0.178 (-0.230; 0.586)	0.384	0.164 (-0.271; 0.599)	0.448	-0.008 (-0.362; 0.345)	0.962	-0.052 (-0.418; 0.314)	0.774	0.338 (-0.161; 0.837)	0.178	0.179 (-0.331; 0.690)	0.479	0.169 (-0.062; 0.399)	0.549	0.157 (-0.081; 0.395)	0.191
Basal plate	-0.023 (-0.388; 0.339)	0.891	0.008 (-0.375; 0.391)	0.966	0.103 (-0.207; 0.413)	0.505	0.112 (-0.263; 0.487)	0.546	-0.055 (-0.367; 0.257)	0.731	-0.062 (-0.375; 0.251)	0.689	0.019 (-0.171; 0.209)	0.840	0.021 (-0.180; 0.221)	0.837
Girls (N=80†)																
PT	0.025 (-0.280; 0.331)	0.868	0.053 (-0.245; 0.350)	0.723	0.110 (-0.211; 0.431)	0.494	0.143 (-0.169; 0.455)	0.360	-0.180 (-0.081; 0.612)	0.129	-0.177 (-0.497; 0.143)	0.270	0.126 (-0.216; 0.467)	0.462	0.112 (-0.239; 0.462)	0.523
Shape	0.026 (-0.246; 0.297)	0.850	-0.039 (-0.310; 0.232)	0.773	0.061 (-0.224; 0.347)	0.667	0.016 (-0.271; 0.302)	0.913	-0.268 (-0.525; -0.012)	0.041*	-0.278 (-0.554; -0.001)	0.049*	-0.206 (-0.494; 0.083)	0.158	-0.198 (-0.498; 0.102)	0.190
Basal plate	-0.057 (-0.368; 0.253)	0.712	-0.098 (-0.406; 0.211)	0.526	0.020 (-0.307; 0.348)	0.900	0.008 (-0.344; 0.361)	0.963	0.011 (-0.323; 0.345)	0.947	0.000 (-0.357; 0.357)	0.999	0.108 (-0.246; 0.462)	0.543	0.177 (-0.192; 0.546)	0.340

Model 1: Adjusted analysis for gestational age.

Model 2: Model 1 + adjusted for maternal age, parity, conception mode, body-mass index and preconception initiation of folic acid supplement use.

*† Number of available measurements for analysis of the placenta and embryo/fetus combined. *Significance at p≤0.05; **Significance at p≤0.01.*

Basal plate = basal plate surface area (mm²); CRL = crown-rump length (mm); EFW = estimated fetal weight (gram); EV = embryonic volume (cm³); PT = placental thickness (mm); Shape = placental ellipsivity.

CHAPTER 5

First-trimester maternal haemodynamic adaptation to pregnancy and placental and embryonic and fetal development: The prospective observational Rotterdam Periconception cohort

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ABSTRACT

Objective: To investigate whether first-trimester maternal haemodynamic adaptation impacts placental, embryonic and fetal development as well as birth outcomes in pregnancies with and without placenta-related complications.

Design: Prospective observational cohort.

Setting: A Dutch tertiary hospital.

Population: 214 ongoing pregnancies.

Methods: At 7-9-11 weeks gestation we assessed maternal haemodynamic adaptation, (mean arterial blood pressure (MAP), uterine artery (UtA) blood flow) and placental development (placental volume (PV), utero-placental vascular volume (uPVV)) using three-dimensional power Doppler ultrasound volumes, and embryonic development (crown-rump length (CRL), embryonic volume (EV)). At 22 and 32 weeks fetal development was assessed by estimated fetal weight. Birth outcomes (birth weight, placental weight) were extracted from medical records. Linear mixed modelling and linear regression analyses were applied.

Main outcome measures: Birth weight centile and placental weight.

Results: In placenta-related complications (n=55, 25.7%), reduced haemodynamic adaptation, i.e., higher UtA pulsatility index (PI) and resistance index (RI) trajectories, was associated with smaller increase in PV (β=-0.559, 95%CI -0.841;-0.278, p<0.001; β=-0.579, 95%CI -0.878;-0.280, p<0.001) and uPVV trajectories (UtA PI: β=-0.301, 95%CI -0.578;-0.023, p=0.034). At birth, reduced haemodynamic adaptation was associated with lower placental weight (UtA PI: β=-0.502, 95%CI -0.922;-0.082, p=0.022; UtA RI: β=-0.435, 95%CI -0.839;-0.032, p=0.036). In pregnancies without placenta-related complications, higher MAP trajectories were positively associated with birth weight centile (β=0.398, 95%CI 0.049;0.748, p=0.025).

Conclusions: Reduced first-trimester maternal haemodynamic adaptation impacts both placental size and vascularization and birth weight centile in particular in pregnancies with placenta-related complications.

INTRODUCTION

Placental development in the first trimester of pregnancy is key to the success of pregnancy and health during the life course[1]. Insufficient spiral artery remodeling and impaired maternal haemodynamic adaptation of the utero-placental vascular network during this early period in pregnancy can result in hypoperfusion of the utero-placental circulation, involved in the causative pathways of placenta-related complications that present from mid-gestation onwards[2-4]. Placenta-related complications include pregnancy-induced hypertension (PIH), preeclampsia (PE), fetal growth restriction (FGR), preterm birth (PTB) and babies born small-for-gestational age (SGA)[5-11].

In clinical practice, maternal haemodynamic adaptation is assessed by mean arterial blood pressure (MAP), determined by cardiac output and systemic vascular resistance, and uterine artery (UtA) blood flow[3]. From the first-trimester onwards, a physiological course of haemodynamic adaptation translates into a decrease in MAP (up to mid-gestation) and resistance to UtA blood flow. Using Doppler ultrasound, UtA blood flow can be measured by the pulsatility index (PI) and resistance index (RI)[12]. Persistent high resistance to UtA blood flow in the second- and third-trimester is positively associated with placenta-related complications[12,13]. From this background we postulate that impaired first-trimester maternal haemodynamic adaptation also increases the risk of adverse early and late placental and embryonic and fetal development.

Previously introduced techniques, such as three-dimensional (3D) ultrasonography combined with Virtual Organ Computer-aided AnaLysis (VOCAL)™, enable the assessment of placental volume (PV)[14]. A smaller first-trimester PV is associated with higher second-trimester UtA resistance, as well as placenta-related complications[15,16]. When Virtual Reality (VR) is combined with 3D power Doppler (PD) ultrasonography, utero-placental vascularization volumes (uPVV) can be measured in a reproducible and accurate manner, using depth perception and offering 3D interaction by creating a hologram from the 3D ultrasound dataset[17-19]. The uPVV measurements were performed on a 3D VR desktop system, allowing for more precise and detailed evaluation of placental structures due to the option of image enlargement, image rotation and the actual use of all 3D dimensions[19]. As such, uPVV in the first trimester can be considered a marker representing maternal haemodynamic adaptation to pregnancy as well as placental development. Here we consider PV and uPVV as surrogate outcomes of placental development only[20].

A longitudinal two-weekly assessment of maternal haemodynamic adaptation to pregnancy as early as from 7 weeks onwards is unique. Moreover, the assessment of associations with placental, embryonic and fetal development this early using 3D Power Doppler ultrasound combined with VOCAL and VR has not been performed previously. Therefore, the overall aim of this study is to investigate associations between the extent of early first-trimester maternal haemodynamic adaptation and early placental development, i.e., PV, uPVV, and embryonic and fetal development, i.e., CRL, EV, EFW, and birth outcomes, i.e., birth weight, placental weight.

METHODS

Study design

This study was part of the Virtual placenta study (registration number Dutch Trial Register: NTR6854), embedded in the ongoing prospective Rotterdam Periconception Cohort (Predict study)[21]. Women were eligible with a singleton pregnancy of less than 10 weeks gestational age (GA). Between January 2017 and March 2018, a total of 241 participants were enrolled. Women were excluded from further analysis in the event of a miscarriage, conception after oocyte donation or withdrawal. At enrolment, participants filled out a questionnaire on general characteristics, medical (and obstetrical) history and lifestyle behaviors. Measurements of height and weight were standardized to calculate body-mass index (BMI) at the first study visit. Geographic origin was categorized as Dutch, Western and Non-Western[22]. Educational level was categorized as low, middle or high according to the classification of Statistics Netherlands[23].

Visits were scheduled at 7, 9, 11, 22 and 32 weeks gestational age (GA). During all visits, blood pressure in mmHg was assessed by a nurse or physician in sitting position, on the right arm, using a manual blood pressure cuff and stethoscope to measure systolic and diastolic blood pressure. The MAP in mmHg was calculated from the systolic blood pressure + 2*diastolic blood pressure, divided by 3.

GA in spontaneous pregnancies was based on the first day of the last menstrual period (LMP) in regular cycles defined as a cycle duration between 25 and 35 days. For pregnancies conceived after in vitro fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI), GA was calculated from the oocyte pick-up day plus 14 days. GA in pregnancies from cryopreserved embryos was defined as the transfer date plus 19 days. GA was based on crown-rump length (CRL) when a difference of more than 6 days existed between the GA based on LMP and the GA based on CRL, or in case of an irregular menstrual cycle or unknown LMP[17].

Ultrasound

At 7, 9 and 11 weeks GA, a transvaginal 3D power Doppler ultrasound volume was obtained to visualize the vascularization of the whole gestational sac including the placenta. At 22 and 32 weeks GA transabdominal ultrasound was performed to estimate fetal growth by parameters needed to calculate estimated fetal weight (EFW) using the Hadlock formula (encompassing the biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL))[24]. At all study visits, pulsed wave Doppler ultrasound was used in a two-dimensional (2D) plane to assess UtA blood flow by determination of the bilateral UtA PI and RI[25].

Ultrasound scans were performed by trained sonographers using a Voluson Expert E8 or E10 ultrasound system with a transvaginal 6-12 MHz transducer (GE, Zipf, Austria) with standard power Doppler settings as described previously (power Doppler gain '-8.0', pulse repetition frequency '0.6 kHz', wall motion filter 'low1', quality 'high')[17]. During 3D scanning, participants were asked to hold their breath for approximately 30 seconds to minimize breathing artefacts. To increase chances of obtaining a complete and high-quality volume, a minimum of two uterine volumes was recorded at a perpendicular angle (90°) to each other.

Total ultrasound scanning time was always limited to 30 minutes per visit according to international safety guidelines[26,27].

Offline 3D measurements

Image quality was scored on a four-point scale ranging between zero and three, based on presence of artefacts (due to fetal movements causing blurring of the volume or acoustic shadowing), the ability to distinct between myometrial and trophoblastic tissue, and completeness of the placenta. For sufficient quality, a score between zero and two was assigned. An incomplete or bad quality volume received a score of three and was excluded.

PV was measured offline using VOCAL by two trained observers according to the following protocol: to measure PV, the circumference of the placenta and embryonic cavity was traced in 2D planes with a 15° rotation step, resulting in a total of 12 placental slices from which a 3D volume was reconstructed. The placental tissue was distinguished from its surroundings by the difference in echogenicity between trophoblast and myometrium. Placental tracing resulted in a total pregnancy volume including the gestational sac. Next, the gestational sac volume was calculated by tracing the gestational sac contours in a similar way as the placenta. The PV in cubic centimeters was then calculated by subtraction of the embryonic cavity volume from the gestational sac volume[15].

uPVV was measured using a VR desktop system with the V-Scope volume rendering application. The VR desktop consists of a 3D monitor, a tracking system for a pointing device, a pair of stereoscopic glasses and a six degrees-of-freedom mouse for 3D volume manipulation. The protocol for uPVV measurements has been shown to be reproducible within and between observers[17]. In short, uPVV was measured by removal of the power Doppler (PD) signal of the embryonic or fetal structures. Then, the difference in echogenicity between the myometrium and placenta was used to erase the PD signal of blood vessels up to the myometrial-placental tissue interface[17].

CRL was measured three times by a length-measuring tool in VR, placing the calipers in a straight line from the crown to caudal rump. By rotating the hologram a correct position of the line in the midsagittal plane was verified. The average was used for analysis. Embryonic volume (EV) was measured once based on grey values by a semi-automated volume-measuring application. The technique and reliability of CRL and EV measurements have been validated previously[28,29].

To determine the relative vascularization degree of the placental bed, the uPVV/PV ratio was calculated.

Definitions of maternal and fetal placenta-related complications

The occurrence of maternal and fetal placenta-related complications was retrieved from medical records. PIH was defined as a systolic blood pressure above 140 mmHg or diastolic blood pressure above 90 mmHg after 20 weeks of gestation without signs of hypertension prior to pregnancy or presence of proteinuria[30]. PE was defined as hypertension with systolic blood pressure above 140 mmHg or diastolic blood pressure above 90 mmHg after 20 weeks of gestation and presence of more than 300 mg proteinuria in a 24 hour period[30,31]. A fetal abdominal circumference and/or estimated fetal weight (EFW) below the 10th centile according to Hadlock curves or a more than twenty-centile decrease on the growth curve, compared to

previous measurements with at least a time span of two weeks, was defined as FGR[32,33]. GA at birth below 37 weeks was defined as preterm birth (PTB) and small-for-gestational-age (SGA) was defined as birth weight below the 10th centile on growth curves specific for GA at birth, parity and fetal gender[30,34].

Statistical analysis

Inherent to the explorative nature of our study we did not perform a power calculation. Moreover, no reference values for the placental measurements in VR are available, making it not possible to determine estimates of expected mean differences needed for power calculation.

Baseline characteristics of the study population were presented as medians with interquartile range or number with percentage. First, to establish normal distributions, data were transformed using a natural log transformation or square root for non-volumetric parameters (MAP, UtA PI, UtA RI and CRL) and cubic root for volumetric parameters (i.e., PV, uPVV, uPVV/PV ratio and EV).

This was followed by a two-step approach to analyze the associations between the haemodynamic and placental parameters. We applied linear mixed models using the individual trajectories of longitudinal measurements (i.e., trajectories of MAP, UtA PI and RI, PV, uPVV, uPVV/PV ratio, CRL, EV and EFW as response variable), with GA as independent variable. In these models we allowed for subject-specific intercepts and slopes (the random effects). Where possible, both the standardized random intercepts and slopes were extracted and used as summaries representing first-trimester haemodynamic adaptation (i.e., MAP, UtA PI and RI). In the next analysis the estimates of first-trimester haemodynamic adaptation were used as covariates in linear regression analyses. Here we used the random effects of the mixed effects models for the trajectories of first-trimester placental development and the trajectories of first-trimester embryonic growth, trajectories of second- and third-trimester EFW, GA at birth, birth weight centile and placental weight as outcomes. Analyses were stratified for pregnancies with and without placenta-related complications. The associations were assessed in a crude model (data not shown). Thereafter, a second model was adjusted for maternal age, parity, conception mode, smoking, fetal gender, BMI and preconception initiation of folic acid supplement use based on the characteristics of the study population and literature. As a last step, sensitivity analyses were performed in strictly-dated pregnancies only.

All analyses were performed using SPSS software (version 25.0; SPSS Inc., Chicago, IL, USA) and RStudio Statistics (version 3.5.0, 2018). P-values <0.05 were considered statistically significant.

RESULTS

A total of 214 ongoing pregnancies out of 241 pregnancies were included in the analysis. Twenty-two women were excluded due to miscarriage, four women were excluded due to oocyte donation and one woman withdrew from the study **(Supplemental Figure 1)**. A total of 466 3D ultrasound datasets were available for further analysis (respectively 101 datasets were
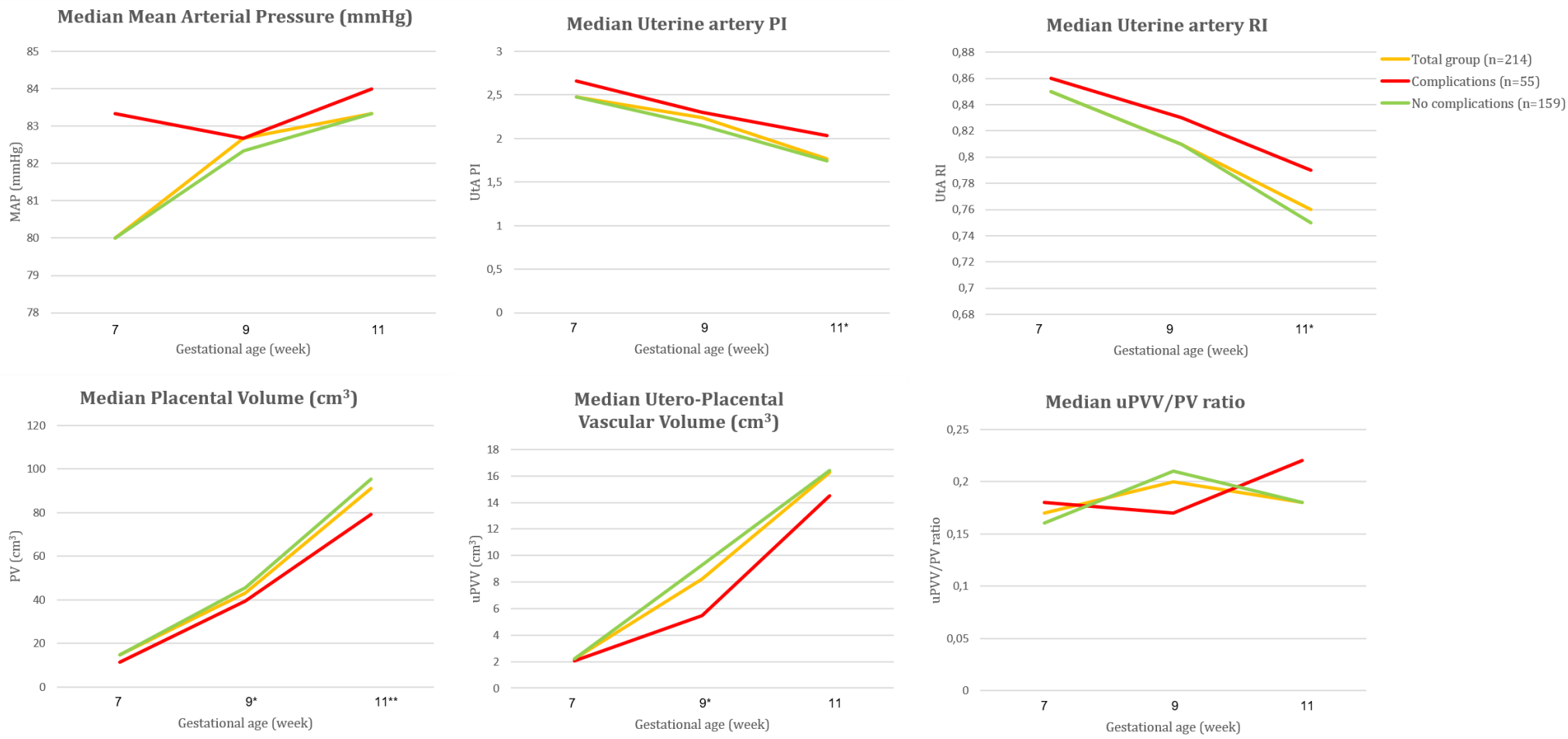
Table 1. Baseline characteristics of the Virtual Placenta study population

Characteristic	All pregnancies (N=214)	Placenta-related complications (N=55)	No placenta related complications (N=159)	*p*-value*
Maternal				
Age, years	32.1 [29.0;35.5]†	30.6 [29.0;34.3]†	32.5 [29.1;35.8]†	0.101
Nulliparous	115 (53.7%)	28 (50.9%)	87 (54.7%)	0.625
Mode of conception, IVF/ICSI	87 (40.7%)	17 (30.9%)	70 (44.0%)	0.088
GA at first visit, days	55 [51;65]†	59 [51;65]†	55 [51;65]†	0.917
Geographic origin				0.453
Dutch	165 (78.9%)	38 (73.1%)	127 (80.9%)	
Western other	6 (2.9%)	1 (1.9%)	5 (3.2%)	
Non-western	38 (18.2%)	13 (25.0%)	25 (15.9%)	
Educational level				0.262
Low	18 (8.6%)	7 (13.5%)	11 (6.9%)	
Intermediate	69 (32.9%)	14 (26.9%)	55 (34.6%)	
High	123 (58.6%)	31 (59.6%)	92 (57.9%)	
BMI measured at first visit in first trimester (kg/m²)	24.9 [22.2;28.4]†	24.8 [22.2;27.5]†	24.0 [21.4;27.0]†	0.235
Folic acid supplement use, yes	210 (98.1%)	54 (98.2%)	156 (98.1%)	0.974
Preconception initiation	175 (81.8%)	40 (72.7%)	135 (84.9%)	0.131
Alcohol consumption, yes	57 (26.6%)	10 (18.2%)	47 (29.6%)	0.100
Smoking, yes	28 (13.1%)	10 (18.2%)	18 (11.3%)	0.193
Birth outcomes				
Major congenital anomalies	8 (3.7%)	0 (0%)	8 (5.0%)	0.090
GA at birth, weeks	39+1 [38+2;40+0]†	38+0 [36+1;39+1]†	39+2 [38+4;40+2]†	**<0.001***
Birth weight, grams	3305 [2930;3565]†	2735 [2329;2931]†	3415 [3175;3685]†	**<0.001***
Placental weight, grams	435 [350;517]†	353 [301;444]†	459 [407;567]†	**<0.001***
Fetal gender, boys	106 (49.5%)	30 (54.5%)	76 (49.7%)	0.535
*Placenta-related complications***	55 (25.7%)	55 (100%)	NA	NA
PIH	9 (4.2%)	9 (16.4%)	NA	NA
PE	7 (3.3%)	7 (12.7%)	NA	NA
FGR	15 (7.0%)	15 (27.3%)	NA	NA
PTB#	23 (10.7%)	23 (41.8%)	NA	NA
SGA	18 (8.4%)	18 (32.7%)	NA	NA

** P-value tested between pregnancies with and without placenta-related complications, significance at p<0.05. †
Expressed as median [interquartile range, p25-p75]. **Overlapping diagnoses. # Iatrogenic and spontaneous preterm
births.*

*BMI = body mass index; FGR = fetal growth restriction, defined as abdominal circumference and/or estimated fetal
weight (EFW) below the 10th centile; GA = gestational age or a more than twenty-centile decrease on the growth
curve, compared to previous measurements with at least a time span of two weeks; ICSI = intracytoplasmic sperm
injection; IVF = in vitro fertilization; NA = not applicable; PE = preeclampsia; PIH = pregnancy-induced hypertension;
PTB = preterm birth; SGA = small-for-gestational age, defined as birth weight below the 10th centile.*

Figure 1. First-trimester maternal haemodynamic parameters and placental parameters, stratified for pregnancies with and without placenta-related complications

*Significance at p≤0.05. ** Significance at p≤0.01*

available at 7 weeks GA, 182 datasets at 9 weeks GA and 183 datasets at 11 weeks GA), of which 328 (70.4%) were usable for VOCAL measurements (respectively 88 datasets were available at 7 weeks GA, 138 datasets at 9 weeks GA and 102 datasets at 11 weeks GA) and 346 (74.2%) for VR measurements (respectively 82 datasets were available at 7 weeks GA, 151 datasets at 9 weeks GA and 113 datasets at 11 weeks GA)[33].

Table 1 shows the study population's baseline characteristics, stratified for pregnancies with and without placenta-related complications. Placenta-related complicated pregnancies (*n*=55, 25.7%) showed a shorter GA at birth (median 38^{+0} vs. 39^{+2} weeks, *p*<0.001), lower birth weight (median 2735 vs. 3415 grams, *p*<0.001) and lower placental weight at birth (median 353 vs. 459 grams, *p*<0.001) compared to the uncomplicated pregnancies (*n*=159, 74.3%).

In placenta-related complicated pregnancies, UtA PI and RI values at 11 weeks GA were higher compared to uncomplicated pregnancies (median PI: 2.03 vs. 1.74, *p*=0.047; median RI: 0.79 vs. 0.75, *p*=0.021). In addition, in placenta-related complicated pregnancies PV (cm^3) and uPVV (cm^3) values were lower at 9 weeks GA (median PV: 39.51 vs. 45.50, *p*=0.028; median uPVV: 5.49 vs. 9.27, *p*=0.018). Furthermore, in these pregnancies PV was lower at 11 weeks GA (median: 79.13 vs. 95.46, *p*=0.002) **(Figure 1 and Supplemental Table 1)**.

First trimester maternal hemodynamic adaptation and first-trimester trajectories of placental development

No significant associations were established between first-trimester maternal haemodynamic adaptation, assessed by MAP trajectories, and placental development **(Table 2)**.

In placenta-related complicated pregnancies, reduced first-trimester maternal haemodynamic adaptation, assessed by an increase in the UtA PI trajectory, was negatively associated with PV trajectories (random intercept adjusted β=-0.559, 95%CI -0.841;-0.278, *p*<0.001) and uPVV trajectories (random intercept adjusted β=-0.301, 95%CI -0.578;-0.023, *p*=0.034). Reduced first-trimester maternal haemodynamic adaptation, assessed by an increase in the UtA RI trajectory, was negatively associated with PV trajectories (random intercept adjusted β=-0.579, 95%CI -0.878;-0.280, *p*<0.001) **(Table 2)**.

In pregnancies without placenta-related complications, reduced first-trimester maternal haemodynamic adaptation, assessed by an increase in the UtA PI and RI trajectories was negatively associated with uPVV trajectories (PI: random intercept adjusted β=-0.303, 95% CI -0.470;-0.136, *p*<0.001, RI: random intercept adjusted β=-0.316, 95% CI -0.483;-0.150, *p*<0.001). Moreover, negative associations were observed for trajectories of the uPVV/PV ratio (random intercept adjusted β=-0.244, 95% CI -0.428;-0.059, *p*=0.010; random slope adjusted β=0.239, 95% CI 0.053;0.425, *p*=0.012) **(Table 2)**.

First-trimester maternal haemodynamic adaptation and trajectories of embryonic and fetal development

No significant associations were identified between maternal haemodynamic adaptation and embryonic and fetal development **(Supplemental Tables 2 and 3)** in pregnancies with or without placenta-related complications.

Table 2. Associations between trajectories of periconceptional maternal haemodynamic adaptation and first-trimester placental trajectories, stratified for cases (placenta-related pregnancy complications) and controls

	1st trimester PV trajectory ($\sqrt[3]{cm^3}$) Random intercept	1st trimester uPVV trajectory ($\sqrt[3]{cm^3}$) Random intercept	1st trimester uPVV/PV ratio trajectory Random intercept	1st trimester uPVV/PV ratio trajectory Random slope
Pregnancies with placenta-related complications (N=55)				
Higher 1st trimester trajectory of MAP				
Random intercept (β), 95% CI	0.154 (-0.772;1.080)	0.036 (-0.796;0.868)	-0.191 (-0.923;0.540)	0.210 (-0.484;0.903)
Random slope (β), 95% CI	-0.343 (-1.262;0.577)	-0.189 (-0.992;0.614)	0.201 (-0.517;0.920)	-0.236 (-0.917;0.445)
Higher 1st trimester trajectory of UtA PI				
Random intercept (β), 95% CI	-0.559 (-0.841;-0.278)**	-0.301 (-0.578;-0.023)*	-0.134 (-0.391;0.122)	-0.034 (-0.282;0.212)
Random slope (β), 95% CI	NA	NA	NA	NA
Higher 1st trimester trajectory of UtA RI				
Random intercept (β), 95% CI	-0.579 (-0.878;-0.280)**	-0.255 (-0.553;0.043)	-0.086 (-0.361;0.190)	-0.084 (-0.347;0.178)
Random slope (β), 95% CI	NA	NA	NA	NA
Pregnancies without placenta-related complications (N=159)				
Higher 1st trimester trajectory of MAP				
Random intercept (β), 95% CI	-0.166 (-0.552;0.220)	-0.229 (-0.617;0.180)	-0.198 (-0.615;0.220)	-0.133 (-0.557;0.291)
Random slope (β), 95% CI	0.230 (-0.161;0.620)	0.203 (-0.197;0.602)	0.112 (-0.313;0.537)	0.134 (-0.297;0.566)
Higher 1st trimester trajectory of UtA PI				
Random intercept (β), 95% CI	-0.090 (-0.260;0.080)	-0.303 (-0.470;-0.136)**	-0.214 (-0.397;0.031)	0.179 (-0.006;0.365)
Random slope (β), 95% CI	NA	NA	NA	NA
Higher 1st trimester trajectory of UtA RI				
Random intercept (β), 95% CI	-0.057 (-0.228;0.114)	-0.316 (-0.483;-0.150)**	-0.244 (-0.428;-0.059)*	0.239 (0.053;0.425)*
Random slope (β), 95% CI	NA	NA	NA	NA

Fully adjusted model for maternal age, parity, conception mode, body-mass index, smoking, preconception initiation of folic acid supplement use and fetal gender. Random slope not available for first-trimester UtA PI, UtA RI, PV and uPVV.

*Significance at p<0.05; **Significance at p<0.01. CI = confidence interval; MAP = mean arterial pressure (in mmHg); NA = not available; PI = pulsatility index; PV = placental volume (in cm³); uPVV = utero-placental vascular volume (in cm³); RI = resistance index; UtA = uterine artery

Table 3. Associations between reduced first-trimester maternal haemodynamic adaptation and birth outcomes, stratified for pregnancies with and without placenta-related complications

	GA at birth (days)	Birth weight centile (p)	Placental weight (grams)
Pregnancies with placenta-related complications (N=55)			
Higher 1st trimester trajectory of MAP			
Random intercept (β), 95% CI	-0.048 (-1.246;1.149)	-0.066 (-0.799;0.668)	-0.900 (-2.302;0.501)
Random slope (β), 95% CI	0.066 (-1.091;1.224)	0.193 (-0.517;0.903)	0.754 (-0.571;2.079)
Higher 1st trimester trajectory of UtA PI			
Random intercept (β), 95% CI	-0.018 (-0.434;0.398)	-0.166 (-0.442;0.090)	-0.502 (-0.922;-0.082)*
Random slope (β), 95% CI	NA	NA	NA
Higher 1st trimester trajectory of UtA RI			
Random intercept (β), 95% CI	0.035 (-0.408;0.479)	-0.234 (-0.503;0.036)	-0.435 (-0.839;-0.032)*
Random slope (β), 95% CI	NA	NA	NA
Pregnancies without placenta-related complications (N=159)			
Higher 1st trimester trajectory of MAP			
Random intercept (β), 95% CI	0.148 (-0.035;0.330)	0.398 (0.049;0.748)*	0.095 (-0.585;0.771)
Random slope (β), 95% CI	-0.174 (-0.362;0.014)	-0.342 (-0.701;0.018)	0.072 (-0.655;0.799)
Higher 1st trimester trajectory of UtA PI			
Random intercept (β), 95% CI	-0.092 (-0.171;-0.012)*	-0.126 (-0.280;0.028)	-0.147 (-0.433;0.140)
Random slope (β), 95% CI	NA	NA	NA
Higher 1st trimester trajectory of UtA RI			
Random intercept (β), 95% CI	-0.110 (-0.187;-0.033)**	-0.107 (-0.258;0.044)	-0.017 (-0.296;0.263)
Random slope (β), 95% CI	NA	NA	NA

Fully adjusted model for maternal age, parity, conception mode, body-mass index, smoking, preconception initiation of folic acid supplement use and fetal gender. Random slope not available for first-trimester UtA PI and UtA RI.

*Significance at p<0.05. ** Significance at p<0.01. CI = confidence interval; GA = gestational age; MAP = mean arterial pressure (in mmHg); NA = not available; PI = pulsatility index; PV = placental volume (in cm³); uPVV = utero-placental vascular volume (in cm³); RI = resistance index; UtA = uterine artery

First-trimester maternal haemodynamic adaptation and birth outcomes
In placenta-related complicated pregnancies, reduced first-trimester maternal haemodynamic adaptation, assessed by an increase in the UtA PI and RI trajectories, only was associated with a lower placental weight (UtA PI random intercept adjusted β=-0.502, 95%CI -0.922;-0.082, *p*=0.022; UtA RI random intercept adjusted β=-0.435, 95%CI -0.839;-0.032, *p*=0.036) **(Table 3)**. No significant associations were revealed between maternal haemodynamic adaptation reflected by MAP and birth outcomes (GA at birth, birth weight centile and placental weight).

In pregnancies without placenta-related complications, reduced first-trimester maternal haemodynamic adaptation, assessed by an increase in the MAP trajectories, was associated with a higher birth weight centile (adjusted β=0.398, 95% CI 0.049;0.748, *p*=0.026). Furthermore, reduced first-trimester maternal haemodynamic adaptation, assessed by an increase in the UtA PI and RI trajectories, was associated with a lower GA at birth (PI: random intercept adjusted β=-0.092, 95% CI -0.171;-0.012, *p*=0.024; RI random intercept adjusted β=-0.110, 95% CI -0.187;-0.033, *p*=0.005) **(Table 3)**.

DISCUSSION

Main findings
We demonstrated that in particular in placenta-related complicated pregnancies, reduced first-trimester maternal haemodynamic adaptation, assessed by higher MAP and UtA blood flow resistance, is associated with a smaller increase in first-trimester placental development, i.e., PV and uPVV. No significant associations were identified between maternal haemodynamic adaptation and embryonic and fetal development. At birth, reduced first-trimester maternal haemodynamic adaptation to pregnancy, as assessed by higher UtA blood flow resistance, is associated with lower placental weight in pregnancies with placenta-related complications. In pregnancies without placenta-related complications, reduced first-trimester maternal haemodynamic adaptation, assessed by an increase in MAP trajectories, was associated with a higher birth weight centile and an increase in UtA PI and RI trajectories, was associated with a shorter GA at birth.

Strengths and limitations
The longitudinal early first-trimester measurements of MAP, UtA PI and RI as markers of maternal haemodynamic adaptation to pregnancy are unique. Unfortunately, it was not possible to estimate both the random intercept and slope for all parameters representing haemodynamic adaptation due to the size of the study population.

Moreover, we used 3D Power Doppler ultrasound combined with VOCAL and VR as an innovative technique to measure PV, uPVV, CRL and EV. At our center there is broad experience in assessment of placental, embryonic and fetal measurements using VR[17]. We observed wide ranges of PV and uPVV values in this population. This is probably due to (patho)physiological differences

in placental development instead of inaccuracies, as these measurements of placental development were proven reproducible within and between observers[17].

Inclusion of participants from as early as 7 weeks gestation is unique in Doppler ultrasound. However, recruitment was performed in a tertiary hospital setting to enable this. The tertiary setting created a high-risk and less generalizable study population, limiting external validity due to a high percentage of IVF/ICSI pregnancies (40.9%), presence of comorbidities and high median age (32 years).

In addition, for reasons of interaction with utero-placental vascular development and inaccuracy of gestational age determination, miscarriages were excluded from analysis. As a downside this could have introduced selection bias since the data may not reflect all early first-trimester pregnancies in the whole general population.

We corrected for a broad range of confounders. Conception mode is relevant since IVF/ICSI pregnancies are more at risk to develop pregnancy complications like FGR and PE[35,36]. It is hypothesized that hormonal treatment in IVF/ICSI pregnancies influences maternal vascular adaptation to pregnancy[37]. We also corrected for parity, since placentas of nulliparous women are on average smaller at birth and nulliparous women more often develop placenta-related complications[38]. Moreover, parity impacts UtA blood flow[39]. We also adjusted for fetal gender since it was demonstrated previously that this modifies first-trimester placental development. However, residual confounding cannot be excluded being inherent to a prospective observational design.

Not all participants were included in the study as early as 7 weeks GA, resulting in missing ultrasound volumes in early pregnancy. Following the critical appraisal of the quality of our 3D ultrasound datasets to ensure appropriate evaluation and generalizability, not all obtained datasets were included for assessment. The highest number of ultrasound volumes was available at 9 weeks GA. Subsequently, beyond 9 weeks GA these numbers were reduced due to increasing quality loss of the acquired ultrasound data or following a large placental size beyond this period. Moreover, obesity and/or uterine position may attenuate image quality. Therefore the included percentage of 3D datasets (70.4% for VOCAL datasets and 74.2% for VR respectively) matched our expectations.

In general, we observed that directions of non-significant effect estimates matched the directions of statistically significant values. Inherent to the explorative and observational design of our study, the likelihood of any Type 1 or Type 2 errors cannot be eliminated. We therefore propose to consider our results most suitable for the purpose of hypothesis-generating and hopefully stimulate future investigations.

Interpretation

We hypothesized that the extent of first-trimester maternal haemodynamic adaptation to pregnancy is associated with first-trimester placental development, and also with fetal development and birth outcome. This is substantiated by our results, showing that in placenta-related complicated pregnancies reduced first-trimester maternal haemodynamic adaptation to pregnancy is associated with less optimal first-trimester placental development and lower placental weight at birth. Available evidence of impaired maternal haemodynamic adaptation and

a higher risk to develop placenta-related complications supports these findings[40-43]. Moreover, previous research confirms that impaired vascular development in PE or FGR in the presence of higher uterine artery Doppler indices is associated with lower serum placental growth factor and lower placental vascularization indices[14,17,44].

Blood pressure and uterine artery blood flow are traditionally considered markers of placental development since increased values with advancing gestation are associated with many placenta-related complications[45]. The direction of causality could be both ways; either less increase in uPVV as a result of reduced maternal haemodynamic adaptation, or reduced maternal haemodynamic adaptation as response to impaired trophoblastic invasion, marked by less development of the utero-placental vasculature. Accumulating evidence is pointing to the first direction, suggesting that changes in maternal uterine and spiral arteries as reflected by UtA Doppler indices are not a direct consequence from trophoblast invasion, but that pre-pregnancy maternal haemodynamics precede placental development[9,46]. In future studies on other periconceptional determinants of maternal haemodynamics can be studied, e.g. cardiac function and small vessel endothelial function. Together they will likely contribute to improved preconception screening and treatment thereby preventing placenta-related complicated pregnancies[47].

Only in placenta-related complicated pregnancies was reduced haemodynamic adaptation associated with a smaller increase in PV trajectories. This suggests that placental tissue and vasculature development are impacted differently and that the impact of first-trimester haemodynamic (mal)adaptation seems more profound in placenta-related complicated pregnancies[46]. Previous studies also describe reduced PV in pregnancies complicated by PE or FGR[48,49].

Earlier work from our group revealed an inverse association between first-trimester maternal vascular risk factors and embryonic size in pregnancies conceived after assisted reproduction[50]. In the current study, reduced first-trimester maternal haemodynamic adaptation was not associated with embryonic or fetal development, possibly since we were challenged with a more restricted availability of embryonic/fetal measurements.

We did retrieve a significant association between reduced first-trimester maternal haemodynamic adaptation and higher birth weighcentile in uncomplicated pregnancies. A first explanation is the uncomplicated nature of this group and that median blood pressure levels remained within normal ranges for pregnancy (**Figure 1**). Second, factors impacting fetal growth during the second- and third-trimester may have had a stronger influence the association between first-trimester maternal haemodynamic adaptation and birth weight centile. Alternatively, the direction of this association could be considered a fetal compensatory phenomenon in response to a more unfavorable utero-placental environment which is substantiated by the developmental origins of health and disease paradigm[10]. A higher birth weight centile was not observed in placenta-related complicated pregnancies, which advocates a reduced capacity to compensate for an unfavorable periconceptional uteroplacental environment.

The association between reduced first-trimester maternal haemodynamic adaptation and lower placental weight in placenta-related complicated pregnancies corresponds with previously

reported findings of lower placental weight and birth weight in pregnancies complicated by PE[51]. Furthermore, first-trimester and postpartum placental size parameters are correlated with each other[52]. We attribute the absence of significance for other birth outcomes to the limited number of placenta-related complications in this cohort and their onset beyond 32 weeks GA. For example PTB comprised spontaneous and iatrogenic cases and the different pathophysiology of early versus late-onset placenta-related complications may have diluted the effect estimates.

Conclusion

This study suggests that reduced first-trimester maternal haemodynamic adaptation to pregnancy, assessed by MAP and UtA blood flow, impairs early placental size and vascularization, as measured by PV and uPVV, and birth weight centile. The impact was different for placenta-related complicated pregnancies. Moreover, median values for MAP and UtA PI and RI were higher in placenta-related complicated pregnancies. While these values were within normal ranges for the first trimester of pregnancy[53], future investigation is substantiated to study which clinical thresholds are sensitive to classify women with a vascular phenotype predisposed to placenta-related complications. This might improve accurate identification of women that can benefit from preventative measures, such as daily acetylsalicylic acid administration, to diminish the risk of developing placenta-related complications. Further, early identification of women at risk allows for implementation of a tailored scheme for antenatal surveillance already from the first trimester onwards. Also the lifestyle intervention www.smarterpregnancy.co.uk and blended lifestyle approach are promising evidence-based interventions[54].

Assessment of first-trimester maternal haemodynamic adaptation and placental development as performed in this study will also enhance future knowledge on the pathophysiology of placenta-related complications. However, the added value of integrating first-trimester parameters of maternal haemodynamic adaptation and PV and uPVV measurements into prediction models for placenta-related complications has to be investigated in larger cohorts first.

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ADDENDUM

Supplemental Figure 1. Flow chart of the Virtual Placenta Study population
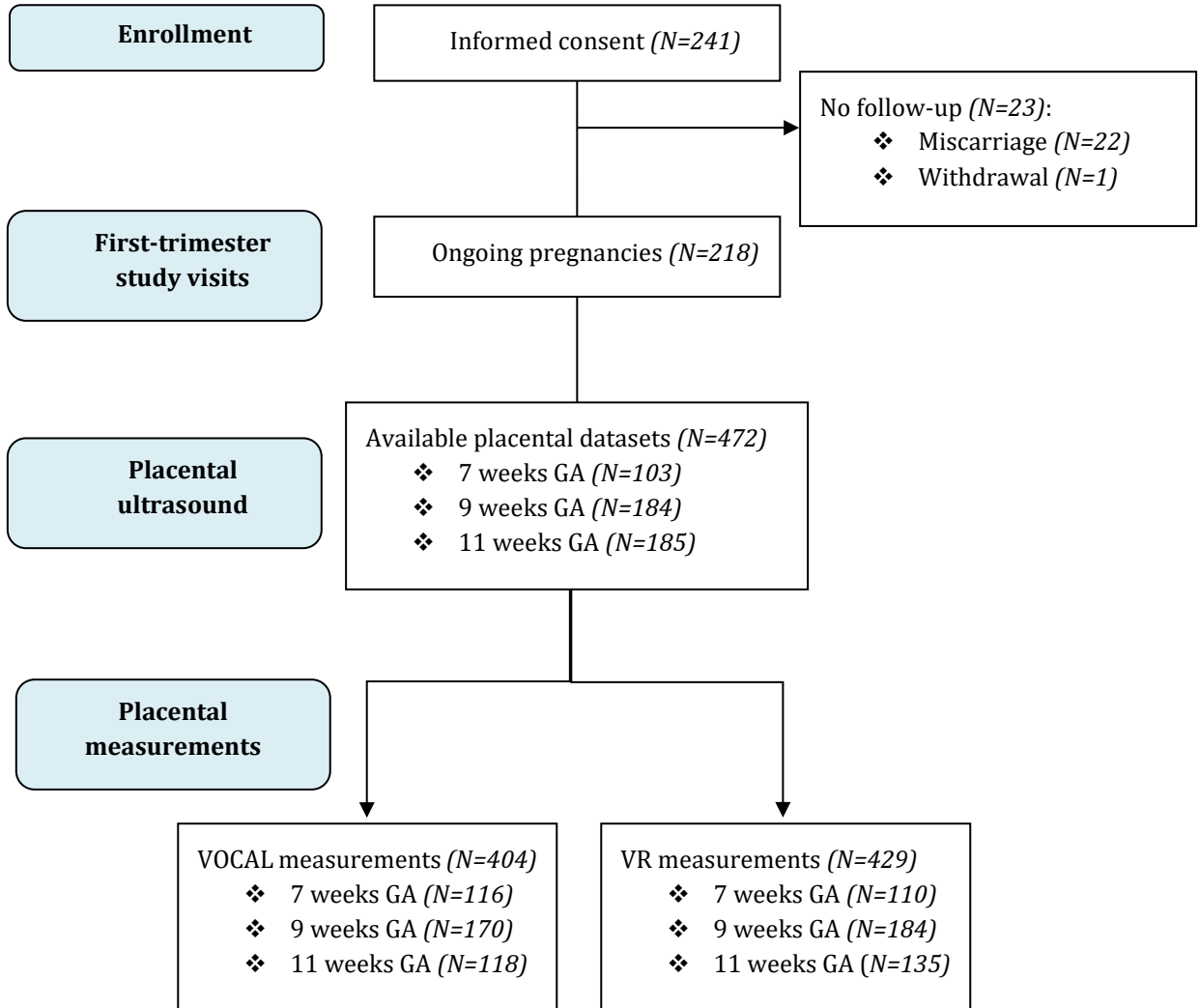
GA = gestational age; VOCAL = Virtual Organ Computer-aided Analyzis; VR = Virtual Reality

Supplemental Table 1. Medians and interquartile ranges of first-trimester maternal haemodynamic adaptation parameters and placental parameters, stratified for pregnancies with and without placenta-related complications

	Week GA	All pregnancies (N=214)	Pregnancies with placenta-related complications (N=55)	Pregnancies without placenta-related complications (N=159)
MAP	7 weeks	80.00 (75.00;86.67)	83.33 (76.67;86.67)	80.00 (75.00;85.67)
	9 weeks	82.67 (76.67;86.67)	82.67 (76.67;90.00)	82.33 (76.83;86.00)
	11 weeks	83.33 (79.33;88.67)	84.00 (79.00;91.66)	83.33 (79.33;88.33)
UtA PI	7 weeks	2.48 (2.11;2.90)	2.66 (2.08;2.96)	2.48 (2.09;2.90)
	9 weeks	2.24 (1.80;2.53)	2.30 (1.89;2.68)	2.15 (1.78;2.51)
	11 weeks	1.77 (1.46;2.27)	2.03 (1.61;2.29)	1.74 (1.39;2.25)
UtA RI	7 weeks	0.85 (0.81;0.89)	0.86 (0.81;0.88)	0.85 (0.81;0.89)
	9 weeks	0.81 (0.76;0.86)	0.83 (0.78;0.87)	0.81 (0.75;0.85)
	11 weeks	0.76 (0.70;0.82)	0.79 (0.73;0.84)	0.75 (0.68;0.81)
PV	7 weeks	14.70 (10.92;20.15)	11.40 (8.95;18.02)	14.82 (11.79;20.17)
	9 weeks	43.17 (32.57;54.48)	39.51 (29.91;49.40)	45.50 (33.35;57.72)
	11 weeks	90.99 (76.45;114.37)	79.13 (60.84;97.53)	95.46 (79.04;118.51)
uPVV	7 weeks	2.20 (1.08;4.43)	2.08 (1.57;4.00)	2.23 (1.05;4.52)
	9 weeks	8.24 (4.37;14.19)	5.49 (3.44;11.37)	9.27 (4.65;15.31)
	11 weeks	16.28 (9.36;25.69)	14.53 (9.33;23.78)	16.42 (9.40;26.32)
uPVV/PV ratio	7 weeks	0.17 (0.10;0.26)	0.18 (0.12;0.21)	0.16 (0.10;0.26)
	9 weeks	0.20 (0.09;0.33)	0.17 (0.08;0.28)	0.21 (0.11;0.37)
	11 weeks	0.18 (0.12;0.28)	0.22 (0.13;0.30)	0.18 (0.12;0.28)

GA = gestational age; MAP = mean arterial pressure (in mmHg); PI = pulsatility index; PV = placental volume (in cm³); uPVV = utero-placental vascular volume (in cm³); RI = resistance index; UtA = uterine artery

Supplemental Table 2. Associations between reduced first-trimester maternal haemodynamic adaptation and trajectories of embryonic development, stratified for pregnancies with and without placenta-related complications

	1st trimester CRL trajectory ($\sqrt{mm}$) Random intercept	1st trimester EV trajectory ($\sqrt[3]{cm^3}$) Random intercept
Pregnancies with placenta-related complications (N=55)		
Higher 1st trimester trajectory of MAP		
Random intercept (β), 95% CI	0.142 (-0.506;0.789)	0.568 (-0.424;1.561)
Random slope (β), 95% CI	-0.029 (-0.666;0.608)	-0.473 (-1.427;0.480)
Higher 1st trimester trajectory of UtA PI		
Random intercept (β), 95% CI	-0.102 (-0.344;0.139)	-0.201 (-0.544;0.129)
Random slope (β), 95% CI	NA	NA
Higher 1st trimester trajectory of UtA RI		
Random intercept (β), 95% CI	-0.139 (-0.404;0.127)	-0.308 (-0.661;0.044)
Random slope (β), 95% CI	NA	NA
Pregnancies without placenta-related complications (N=159)		
Higher 1st trimester trajectory of MAP		
Random intercept (β), 95% CI	0.193 (-0.249;0.636)	-0.015 (-0.397;0.367)
Random slope (β), 95% CI	0.051 (-0.391;0.492)	0.123 (-0.263;0.508)
Higher 1st trimester trajectory of UtA PI		
Random intercept (β), 95% CI	0.004 (-0.205;0.212)	0.160 (-0.008;0.327)
Random slope (β), 95% CI	NA	NA
Higher 1st trimester trajectory of UtA RI		
Random intercept (β), 95% CI	-0.027 (-0.223;0.168)	0.129 (-0.038;0.296)
Random slope (β), 95% CI	NA	NA

Fully adjusted model for maternal age, parity, conception mode, body-mass index, smoking, preconception initiation of folic acid supplement use, fetal gender and first-trimester placental parameters (PV, uPVV and uPVV/PV ratio). Random slope not available for first-trimester UtA PI, UtA RI and CRL.

CI = confidence interval; CRL = crown-rump length (in mm); EV = embryonic volume (in cm³); GA = gestational age; MAP = mean arterial pressure (in mmHg); NA = not available; PI = pulsatility index; PV = placental volume (in cm³); uPVV = utero-placental vascular volume (in cm³); RI = resistance index; UtA = uterine artery

Supplemental Table 3. Associations between reduced first-trimester maternal haemodynamic adaptation and fetal development, stratified for pregnancies with and without placenta-related complications

	2nd/3rd trimester EFW trajectory (grams) Random intercept	2nd/3rd trimester EFW trajectory (grams) Random slope
Pregnancies with placenta-related complications (N=55)		
Higher 1st trimester trajectory of MAP		
Random intercept (β), 95% CI	0.739 (-0.009;1.487)	-0.752 (-1.628;0.125)
Random slope (β), 95% CI	-0.710 (-1.436;0.015)	0.840 (-0.009;1.690)
Higher 1st trimester trajectory of UtA PI		
Random intercept (β), 95% CI	--0.043 (-0.310;0.224)	0.057 (-0.255;0.370)
Random slope (β), 95% CI	NA	NA
Higher 1st trimester trajectory of UtA RI		
Random intercept (β), 95% CI	0.007 (-0.279;0.294)	-0.048 (-0.381;0.284)
Random slope (β), 95% CI	NA	NA
Pregnancies without placenta-related complications (N=159)		
Higher 1st trimester trajectory of MAP		
Random intercept (β), 95% CI	-0.088 (-0.498;0.321)	0.342 (-0.013;0.697)
Random slope (β), 95% CI	0.072 (-0.335;0.489)	-0.308 (-0.666;0.049)
Higher 1st trimester trajectory of UtA PI		
Random intercept (β), 95% CI	0.129 (-0.049;0.308)	-0.141 (-0.297;0.016)
Random slope (β), 95% CI	NA	NA
Higher 1st trimester trajectory of UtA RI		
Random intercept (β), 95% CI	0.092 (-0.082;0.266)	-0.117 (-0.271;0.037)
Random slope (β), 95% CI	NA	NA

Fully adjusted model for maternal age, parity, conception mode, body-mass index, smoking, preconception initiation of folic acid supplement use, fetal gender and first-trimester placental parameters (PV, uPVV and uPVV/PV ratio). Random slope not available for first-trimester UtA PI and UtA RI.

CI = confidence interval; EFW = estimated fetal weight; MAP = mean arterial pressure (in mmHg); NA = not available; PI = pulsatility index; PV = placental volume (in cm³); uPVV = utero-placental vascular volume (in cm³); RI = resistance index; UtA = uterine artery

CHAPTER 6

Larger first-trimester placental volumetric parameters are associated with lower pressure and more flow-mediated vasodilation of the feto-placental vasculature after delivery

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ABSTRACT

Objective: To explore the correlation between *in-vivo* placental volumetric parameters in the first trimester of pregnancy and *ex-vivo* parameters of feto-placental vascular function after delivery.

Methods: In ten singleton physiological pregnancies, placental volume (PV) and utero-placental vascular volume (uPVV) were measured offline in three-dimensional ultrasound volumes at 7, 9 and 11 weeks gestational age (GA) using Virtual Organ AnaLysis and Virtual Reality. Directly postpartum, term placentas were *ex-vivo* dually perfused and pressure in the feto-placental vasculature was measured to calculate baseline pressure (pressure after a washout period), pressure increase (pressure after a stepwise fetal flow rate increase of 1 mL/min up to 6 mL/min) and flow-mediated vasodilation (FMVD; reduction in inflow hydrostatic pressure on the fetal side at 6mL/min flow rate). Correlations between *in-vivo* and *ex-vivo* parameters were assessed by Spearman's correlation coefficients (R).

Results: Throughout the first trimester, PV was negatively correlated with pressure increase (R_{growth}=-0.84) and, at 11 weeks GA, also positively correlated with FMVD (R=0.89). At 7 weeks GA, uPVV and uPVV/PV ratio were negatively correlated with pressure increase (R=-0.58 and R=-0.81, respectively) and positively correlated with FMVD (R=0.62 and R=0.90, respectively).

Discussion: Mainly in the early first trimester, larger placental volumetric parameters are associated with lower pressure and more FMVD in the feto-placental vasculature after delivery. This may suggest that larger and/or more vascularized placentas in early pregnancy have better adaptive mechanisms and possibly lead to better pregnancy outcomes.

INTRODUCTION

Placenta-related pregnancy complications, such as preeclampsia (PE) and fetal growth restriction (FGR), are highly prevalent and not only affect fetal development and pregnancy outcome, but also future maternal and offspring health[1-3]. Most of these pregnancy complications originate already in the first trimester of pregnancy[4]. Within this time window, development of the placental bed takes place, which is characterized by remodelling of the uterine spiral arteries, thereby creating a low-resistance circulation. Adequate remodelling is crucial to placental development, subsequently affecting embryonic and fetal health[5,6]. After the placental vascular network has been formed in early pregnancy, capillary growth continues until delivery, mediated by various growth factors. From mid-gestation onwards, there is an exponential growth in vascular volume of feto-placental vessels to accommodate the needs of the growing fetus[7].

Non-invasive assessment of *in-vivo* placental development remains challenging, since the value of available markers of placental function and development is limited. Most commonly, placental function is assessed by the use of derivatives of the placental circulation. For example, abnormal uterine artery Doppler waveforms have been related to pregnancy complications, such as pregnancy-induced hypertension and PE[8,9]. An innovative method to determine placental development resulted from the introduction of Virtual Organ Computer-aided AnaLysis (VOCAL), which enables the assessment of three-dimensional (3D) placental volume measurements and utero-placental vascularisation indices (i.e. vascularisation indices, flow indices and vascularisation-flow indices). These parameters have all been associated with adverse outcomes, such as miscarriage, PE and FGR[10-12]. A newly developed technique is Virtual Reality (VR) which can be combined with measurements of 3D power Doppler ultrasound volumes and visualizes the placental circulation from early pregnancy onwards, in three dimensions with depth perception. As previously demonstrated by Reijnders *et al.* this technique is feasible and reliable for use in the first trimester of pregnancy to measure placental parameters, that reflect placental volume and utero-placental vascularisation of the uterine/maternal side (i.e. the placental bed)[13].

Ex-vivo assessment of the feto-placental vasculature can be performed using dual-sided placental perfusion, an experimental model to study fetal vascular reactivity of a single cotyledon directly after birth. Unlike most other vascular systems, the feto-placental vasculature is not innervated. Local vascular tone and fetal cardiac output are the main determinants of blood flow through these vessels, regulated by circulating and locally produced hormones and vasoactive compounds[14]. Therefore, flow-mediated pressure changes in the *ex-vivo* dual-sided perfused cotyledon are a measure of vascular resistance in the placenta. Jones *et al.* have already shown that there is a significant correlation between vascular resistance measured *in-vivo* (i.e. umbilical artery Doppler velocimetry at term) and *ex-vivo* placental perfusion[15]. However, no study has yet assessed the relation between *in-vivo* parameters of early placental vascular development and *ex-vivo* placental vascular perfusion.

Since early non-invasive assessment of placental development is challenging and it is unknown whether available markers actually represent placental function later in pregnancy, the aim of this study was to explore whether correlations exist between *in-vivo* ultrasound parameters of first-trimester placental (vascular) development and *ex-vivo* parameters of feto-

placental vascular reactivity at delivery. Not only will this provide better insight in the pathophysiology of placental disorders, it could also demonstrate the need for earlier evaluation of the placental circulation.

METHODS

This explorative study was conducted within the Virtual placenta study, embedded in the Rotterdam Periconception Cohort (Predict Study), which is an ongoing prospective cohort study performed at the Department of Obstetrics and Gynecology of the Erasmus MC, University Medical Center in Rotterdam, The Netherlands[16]. Women who participated in the Predict study before 10 weeks gestational age (GA), were also invited to participate in the Virtual Placenta study that was carried out between January 2017 and March 2018. Pregnancies conceived either spontaneously or through assisted reproduction techniques (ART) were eligible. The study protocol has been approved by the Erasmus MC Institutional Review Board (MEC 2015-494). All participating women and their partners signed written informed consent at enrolment, also on behalf of their unborn child. Women were asked for consent to use their placenta for research purposes after delivery. Women with multiple pregnancies, (gestational) diabetes, viral infections (e.g. HIV) or placental anomalies were not eligible for inclusion in this subset.

Study Parameters
Maternal characteristics were obtained from self-reported questionnaires filled out upon enrolment. First-trimester body-mass index (BMI) and blood pressure were also measured at intake. After delivery, participating women again filled out a questionnaire on pregnancy and birth outcomes. The retrieved information was checked with data from medical records and delivery reports where available.

Ultrasound
Participants underwent serial 3D ultrasound examinations at 7, 9 and 11 weeks GA to obtain volumes encompassing the whole embryo and placenta. Ultrasound examinations were performed by experienced sonographers only, using a Voluson E8 or E10 system (GE Medical Systems, Zipf, Austria). In the first trimester, 3D ultrasound examinations were performed using a transvaginal 6-12 MHz transducer. Vasculature of the complete placenta and embryo was imaged using Power Doppler (PD) US with standardized setting (PD gain '-8.0', pulse repetition frequency (PRF) '0.6 kHz', wall motion filter (WMF) 'low1', quality 'high'). To minimize artefacts and measurement errors by movement, participants were asked to hold their breath for approximately 30 seconds during image acquisition. All ultrasound examinations were performed according to international guidelines on safe use of Doppler ultrasound in the first trimester of pregnancy (ALARA-principle) and, as such, total scanning time was kept as low as possible with a maximal duration of 30 minutes and a maximal thermal index of 1.3[13,17-19].

Offline 3D measurements

Placental volume (PV) measurements were performed with offline specialized VOCAL software (4D View, GE Medical System) according to standardized methods, to reconstruct the trophoblast[11]. Utero-placental vascular volume (uPVV) was measured using a VR desktop system (**Figure 1**). VR enables visualization of a 3D volume as a true hologram, which allows for depth perception and thus more optimal assessment of the utero-placental vascularization. The VR desktop is a validated system composed of a personal computer using the V-Scope volume rendering application, a two-dimensional (2D) monitor which displays the user interface, a 3D monitor to display the volume, a tracking system allowing for interaction of the observer with the 3D volume, a pair of stereoscopic glasses to enable depth perception and a six-degrees of freedom mouse for 3D volume manipulation[20]. By thresholding the 8-bit (range 0-255) Doppler magnitude data,

semi-automatic volume measurements of the uPVV were obtained. To enable the most optimal visualization of the utero-placental vasculature, the lower-Doppler threshold level was set at a value of 100, which means that semi-automatic calculations only color and count by voxels with a Doppler value of 100 or higher. First, embryonic structures were removed from the segmentation. Then, the difference in echogenicity between the myometrium and placenta was used to erase the vessels up to the myometrial-placental border, thereby leaving the maternal vascularization of the utero-placental bed for volume assessment. Currently it is not possible to distinct between the maternal blood space and embryonic vasculature within the uPVV. A more detailed description and validation of the methods for uPVV measurements has previously been published[13]. After VOCAL and VR measurements, uPVV was divided by PV to calculate a placental vascular volume ratio (uPVV/PV ratio). Due to limited image quality or study inclusion after 7 weeks GA, measurements of PV and uPVV could not be performed for all included pregnancies and/or study visits.

Figure 1. Visualization of a three-dimensional power Doppler utero-placental vascular volume in Virtual Reality
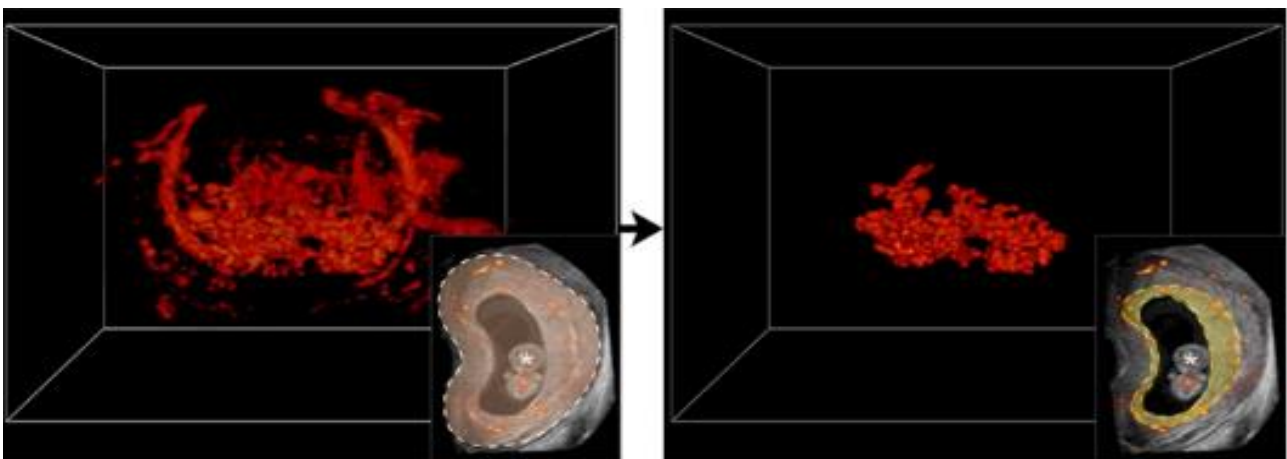

On the left; complete three-dimensional power Doppler (3D PD) vascular volume at 9 weeks of gestation. On the right; Using grey values of the utero-placental tissue, a virtual brush allows to erase vascular voxels up to the myometrial-placental tissue interface margin, leaving utero-placental vascular volume (uPVV) to be measured by threshold-based segmentation. Lower inserts; two-dimensional power Doppler (2D PD) image with complete vasculature in color delineated by dashed white line (left), placental vascularization delineated by dashed yellow line (right).

Placental perfusion

The perfusion model used in our study has been previously described in detail by Hitzerd *et al.*[21]. In short, term placentas were collected immediately after delivery and fetal circulation was established by cannulating a corresponding chorionic artery and vein of an intact cotyledon. Fetal flow rate was started at 1 mL/min. When cannulation was successful, the cotyledon was cut from the placenta and placed inside the perfusion chamber. Maternal circulation (constant flow rate of 12 mL/min) was created by placing four blunt cannulas in the intervillous space. Venous outflow was collected in a reservoir underneath the cotyledon and run back to the maternal reservoir. Perfusion media consisted of Krebs-Henseleit buffer, supplemented with heparin (5000 IU, 0.5 mg/L) and aerated with 95% O_2 – 5% CO_2. A placental washout period of approximately 30 minutes was performed before starting an experiment. Changes in pressure on the fetal side of the placenta were measured by pressure transducers and recorded throughout the experiment using acquisition software (Biopac, Goleta, CA USA). When a stable baseline pressure was reached after the washout period, the fetal flow rate was increased stepwise with 1 mL/min, until a flow rate of 6 mL/min was reached. After each step a new steady state in pressure was awaited before continuing with the next step (**Figure 2**). The parameters baseline pressure, total pressure increase and flow-mediated vasodilation (FMVD) were used for analysis. Total pressure increase was defined as the difference between baseline at pressure at start of the experiment and the new steady state at a flow rate of 6 mL/min. As previously described by Jones *et al.*, FMVD is the percentage of reduction in hydrostatic pressure on the fetal side as a result of increased flow rate, measured at a flow rate of 6 mL/min[15].

Figure 2. Stepwise increase in fetal flow rate leading to increase in pressure (representative)
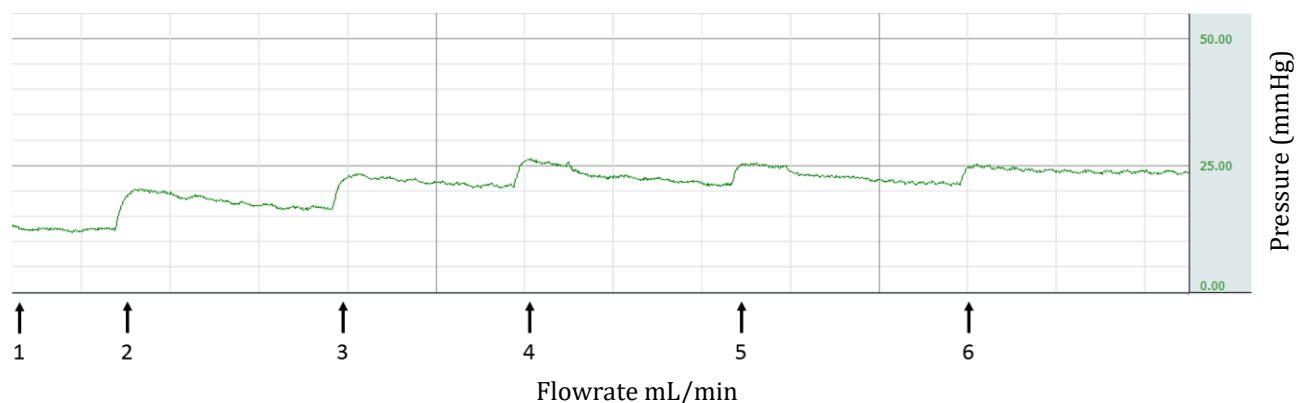
Statistical analysis

Because of skewed distributions of most parameters, data are presented as medians (interquartile range). To identify correlations between *in-vivo* and *ex-vivo* measurements, Spearman's rank correlation coefficients (R-values) were used and correlations were plotted in scatterplots. To further evaluate these correlations, linear mixed models were used to calculate individual slopes for each participant to establish placental growth trajectories throughout the first trimester (at 7, 9 and 11 weeks GA). In these models, uPVV, PV and the uPVV/PV ratio were transformed using a cubic root. The individual slopes were then also correlated with *ex-vivo* parameters using Spearman's correlation coefficients. All analyses were performed using SPSS

software (version 21.0; SPSS Inc., Chicago, IL, USA) and RStudio Statistics (version 3.5.0, 2018). Correlations >0.5 were considered relevant and *P*-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics
In this explorative study, twelve women were included, of whom ten placentas were successfully perfused. Baseline characteristics of these ten women are provided in **Table 1**. Women had a median age of 31.9 years (29.7-37.1), 40% were nulliparous and 90% conceived spontaneously. None of the women smoked or used alcohol during pregnancy. In 60% of the women, the mode of delivery was an elective caesarean section (two nulliparous – and four multiparous women), all because a of breech position and/or previous caesarean section. Two women delivered spontaneously and another two women underwent an emergency caesarean because of failure to progress. Median birth weight was 3365 grams (2835-3425) and 70% of the offspring was male. None of the pregnancies were complicated by PE or any other pregnancy complication. All infants were born at term (i.e. >37 weeks GA), and one infant was small-for-gestational-age (birth weight below the 10th centile)[22]. Median placental weight was 395 grams (322-451), and of two placentas, weight was below the 10th centile.

Placental vascular measurements
For the ten included pregnancies, six women had multiple measurements available for PV and eight women had multiple measurements available for uPVV. Three women had measurements available at all weeks for PV, four women had available measurements at all weeks for uPVV. A total of five measurements of PV and uPVV were available at 7 weeks GA, eight measurements were available of PV and nine of uPVV at 9 weeks GA, and five measurements were available of PV and seven of uPVV at 11 weeks GA. Median values for *in-vivo* placental measurements per week GA are displayed in **Table 2a** and increased from a median of 3.26 cm^3 (0.96-6.40) at 7 weeks GA to 13.36 cm^3 (6.27-30.08) at 11 weeks GA for uPVV, a median of 20.15 cm^3 (12.95-23.90) at 7 weeks GA to 92.76 cm^3 (69.18-125.36) at 11 weeks GA for PV, and a median of 0.16 (0.08-0.23) at 7 weeks GA to 0.17 (0.10-0.35) at 11 weeks GA for the uPVV/PV ratio.

Placental perfusion
Median gestational age at delivery was 38^{+5} (37^{+4}-39^{+1}) weeks. Median values for *ex-vivo* placental measurements are displayed in **Table 2b**. Median baseline pressure at the starting flow rate of 1 mL/min was 21 mmHg (19-25) mmHg, which increased to 32.5 mmHg (23.8-36.3) at 6 mL/min, leading to a total pressure increase of 10.5 mmHg (3.0-13.0) (**Figure 3a and 3b**). Median FMVD at 6 mL/min was 50% (50-100) (**Figure 3c**).

Table 1. Baseline characteristics (N=10)

Characteristics	
Maternal	
Age at intake (years)	31.9 (29.7-37.1)
Nulliparous	4 (40%)
Mode of conception	
- Spontaneous	9 (90%)
- IVF/ICSI	1 (10%)
Geographic origin	
- Dutch	7 (70%)
- Western	1 (10%)
- Non-western	2 (20%)
Educational level	
- Low	0 (0%)
- Intermediate	3 (30%)
- High	7 (70%)
BMI, first-trimester (measured)	22.8 (21.7-32.5)
Periconceptional folic acid supplement use	10 (100%)
Median first trimester RR (intake)	
- Systolic	110.0 (103.0-114.0)
- Diastolic	65.5 (63.5-68.5)
Periconceptional smoking	1 (10%)
Periconceptional alcohol use	0 (0%)
At delivery	
Gestational age at delivery	38^{+5} (37^{+4}-39^{+1})
Mode of delivery	
- Vaginal	2 (20%)
- Elective caesarean	6 (60%)
- Emergency caesarean	2 (20%)
Placental weight	395 (322-451)
Placental weight <p10 at birth	2 (20%)
Histology: distal villous hypoplasia	1 (10%)
Neonatal	
Birth weight	3365 (2835-3425)
Small for gestational age	1 (10%)
Sex	
- Male	7 (70%)
- Female	3 (30%)

Data are expressed as median (interquartile range) or number (percentage).

Table 2a. Median and ranges of measurable *in-vivo* placental volumetric parameters per week GA

	7 weeks GA (N=5)		9 weeks GA (N=9)		11 weeks GA (N=7)	
	Median	*IQR*	*Median*	*IQR*	*Median*	*IQR*
PV (cm^3)	20.15	12.95 - 23.90	44.96	25.57 - 57.25	92.76	69.18 - 125.36
uPVV (cm^3)	3.26	0.96 - 6.40	8.80	3.97 - 10.61	13.36	6.27 – 30.08
uPVV/PV ratio	0.16	0.08 - 0.23	0.16	0.14 - 0.33	0.17	0.10 - 0.35

uPVV = utero-placental vascular volume (in cm^3); PV = placental volume (in cm^3); uPVV/PV ratio = ratio between uPVV and PV; IQR = interquartile range

Table 2b. Median and ranges of *ex-vivo* placental measurements at term

N=10	*Median*	*IQR*
Pressure at baseline	21.0	19.0 - 25.0
Total pressure increase (mmHg)	10.5	3.0 - 13.0
End pressure (mmHg)	32.5	23.8 - 36.3
FMVD at 6 ml/min	50.0	50.0 - 100.0

FMVD = flow-mediated vasodilation (% pressure reduction from peak to new steady state); IQR = interquartile range

Figure 3. *Ex-vivo* placental perfusion parameters
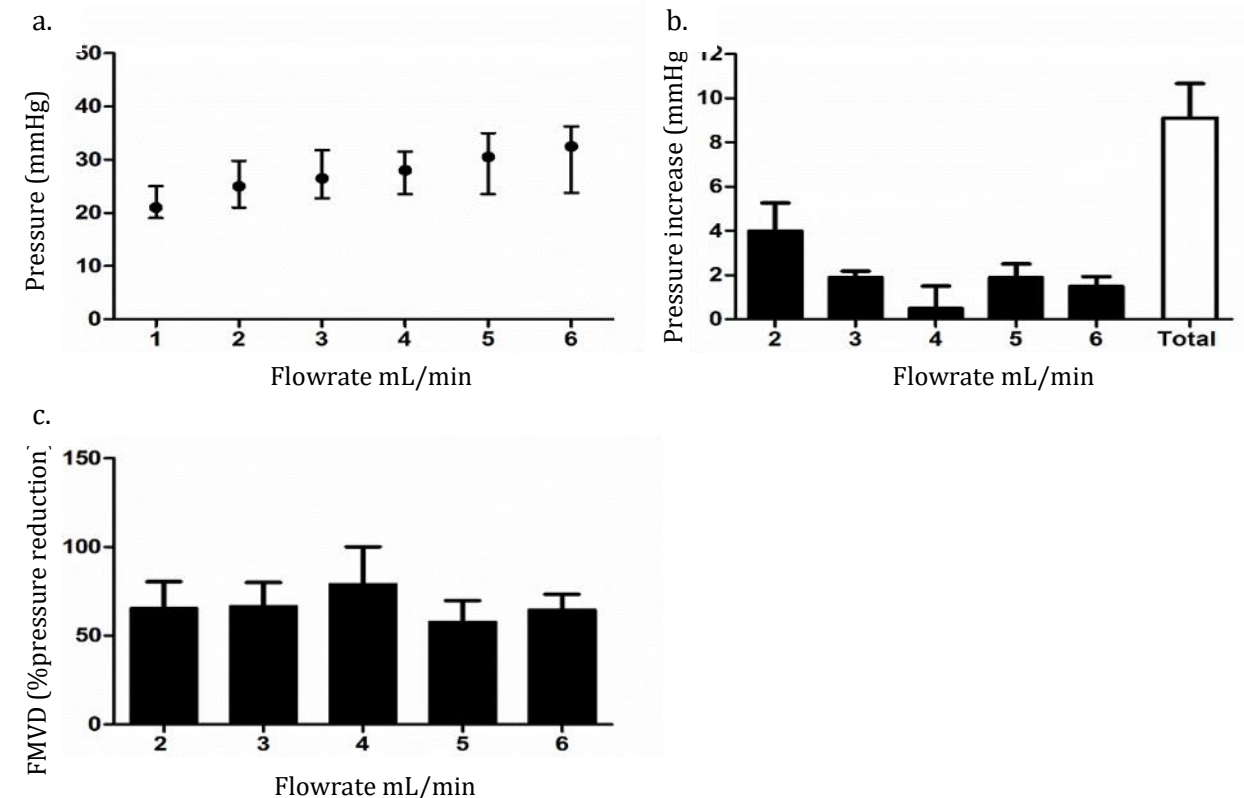
Panel a shows the steady state feto-placental pressure that was measured after each increase of flow rate. Pressure increase was highest after the first increase of flow rate from 1 to 2 mL/min (panel b). Panel c shows the flow-mediated vasodilation (FMVD) per flow rate. Data (n=10) are shown as median (interquartile range).

Correlations

Correlations between *in-vivo* and *ex-vivo* measurements are depicted in **Table 3** and **Figure 4**. PV was negatively correlated with pressure increase at 7 weeks GA (R=-0.53), 9 weeks GA (R=0.74) and 11 weeks GA (R=-0.98). At 11 weeks GA, PV was positively correlated with baseline pressure (R=0.62) and FMVD (R=0.89). uPVV was negatively correlated with pressure increase (R=-0.58) and positively correlated with FMVD (R=0.62) at 7 weeks GA. No correlations between uPVV and *ex-vivo* parameters were observed at 9 and 11 weeks GA. The uPVV/PV ratio was negatively correlated with pressure increase (R=-0.81) and positively correlated with FMVD (R=0.90) at 7 weeks GA. At 11 weeks GA, a negative correlation was

observed between the uPVV/PV ratio and FMVD (R=-0.67), although this correlation was not statistically significant.

When studying the correlations between *in-vivo* placental growth throughout the first trimester (i.e. slopes of placental parameters) and cross-sectional *ex-vivo* placental parameters, a significantly negative correlation was observed between first-trimester PV growth and FMVD (R_{growth}=-0.84) and between uPVV/PV ratio and FMVD (R_{growth}=-0.90). Also, relevant positive correlations, although not statistically significant, were observed between PV growth and FMVD (R_{growth}=0.50) and between uPVV/PV ratio growth and pressure increase (R_{growth}=0.51).

**Table 3.** Correlations between *in-vivo* and *ex-vivo* placental parameters per week GA

Placental parameter		R_{7weeks}	R_{9weeks}	$R_{11weeks}$	R_{growth}
PV	- pressure at baseline	-0.05	-0.05	0.62	0.25
	- total pressure increase	-0.53	-0.74*	-0.98*	-0.84*
	- FMVD at 6ml/min	0.05	-0.04	0.89*	0.50
uPVV	- pressure at baseline	0.15	-0.40	0.42	0.03
	- total pressure increase	-0.58	0.14	0.22	-0.25
	- FMVD at 6ml/min	0.62	-0.25	-0.21	0.15
uPVV/PV ratio	- pressure at baseline	-0.24	-0.01	0.05	-0.15
	- total pressure increase	-0.81	0.12	0.36	0.51
	- FMVD at 6ml/min	0.90*	0.21	-0.67	-0.90*

*R= Spearman's correlation coefficient. Relevant correlations in bold. *Significant at level <0.05.*
FMVD = flow-mediated vasodilation (% pressure reduction from peak to new steady state); uPVV = utero-placental vascular volume (in cm³); PV = placental volume (in cm³); uPVV/PV ratio = ratio between uPVV and PV; R = correlation coefficient.

Figure 4. Scatterplots depicting correlations between *in-vivo* and *ex-vivo* placental parameters
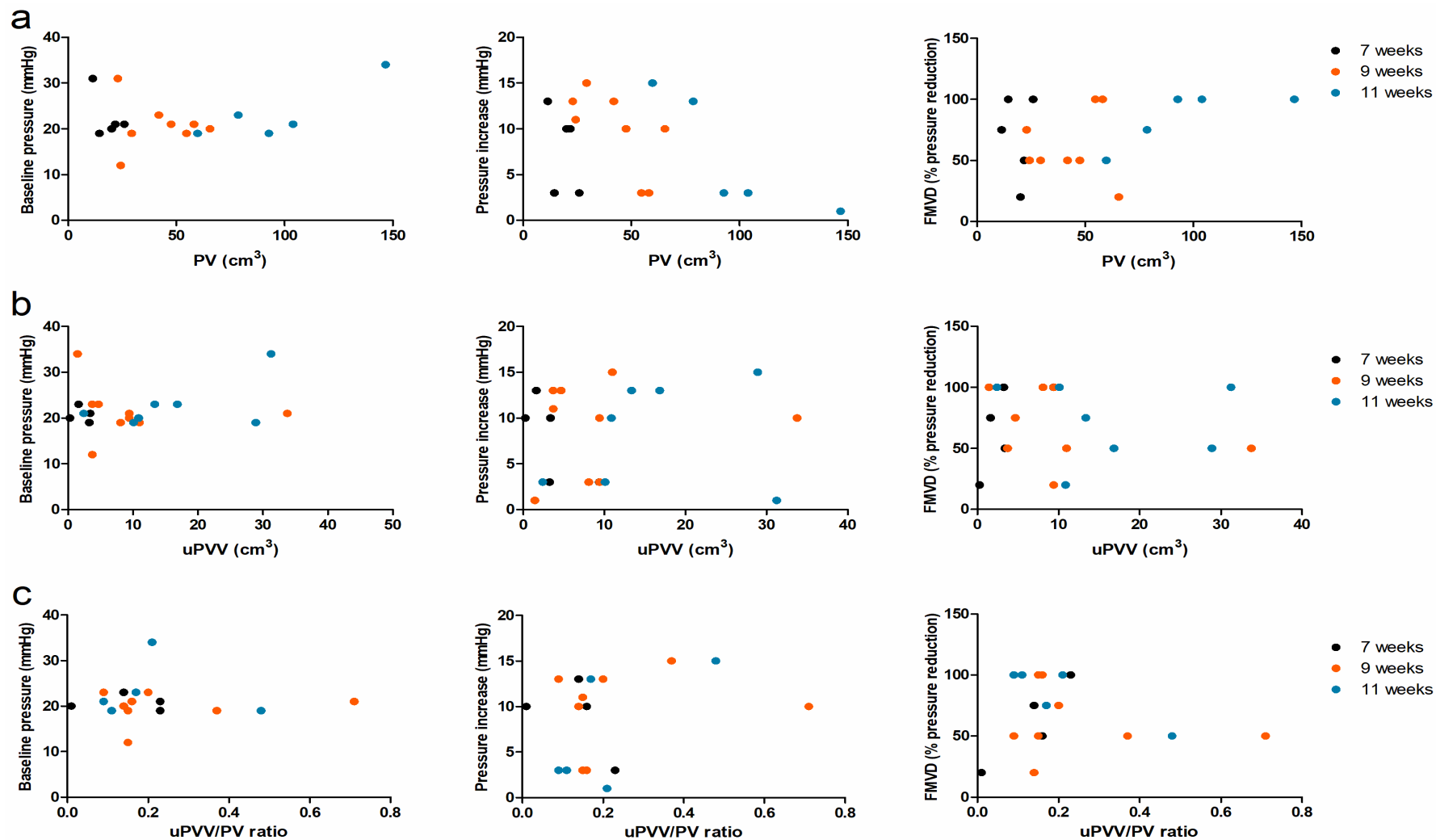
This figure shows the correlations of the ex-vivo parameters measured postpartum and PV (panel a), uPVV (panel b) and the uPVV/PV ratio (panel c) at 7 weeks GA (black circles), 9 weeks GA (orange circles) and 11 weeks GA (blue circles).

FMVD = flow-mediated vasodilation (% reduction in pressure from peak to new steady state); PI = pressure increase; PV = placental volume (in cm³); uPVV = utero-placental vascular volume (in cm³); uPVV/PV ratio = ratio between uPVV and PV.

DISCUSSION

The results of this study suggest that, mainly in the early first trimester, larger placental volumetric parameters, measured by 3D ultrasound and VR technique, are associated with lower pressure and more FMVD in the feto-placental vasculature after delivery. Correlations between *in-vivo* placental growth throughout the first trimester and *ex-vivo* placental parameters were negative for the growth of PV and pressure increase (R=-0.84), but positive for FMVD (R=0.50) although not statistically significant. In contrast, a negative correlation existed between the growth of first-trimester uPVV/PV ratio and FMVD (R= -0.90).

To our knowledge, the current study is the first to investigate associations between *in-vivo* first-trimester ultrasound parameters of the maternal utero-placental circulation and third-trimester *ex-vivo* perfusion parameters of the feto-placental circulation. Previously, it has been shown by Jones *et al.* that at term there is a positive correlation between *in-vivo* umbilical artery Doppler velocimetry and *ex-vivo* feto-placental vascular resistance in placentas from uncomplicated pregnancies[15]. However, one should keep in mind that umbilical artery Doppler velocimetry and *ex-vivo* vascular resistance both represent the fetal side of the placenta, while our placental parameters reflect the volume and vascularization of the uterine/maternal side (i.e. the utero-placental bed). In line with the results of Jones *et al.*, we found a negative correlation between pressure increase at *ex-vivo* perfusion and PV (throughout the first trimester) and uPVV (at 7 weeks GA only). Furthermore, this corresponds with the positive correlation that was observed between PV, uPVV and FMVD. These findings suggest a greater ability of larger placentas to adjust to higher pressure by vasodilation, due to a greater compensatory capacity in the form of vasodilation. This is contrasted by the finding that the growth trajectory of uPVV/PV ratio was positively correlated with pressure increase and negatively with FMVD, which suggests that placentas with more vascular development (i.e. more increase of uPVV compared to PV throughout the first trimester) demonstrate higher pressure and less vasodilation in response to flow. Since PV and uPVV only reflect the maternal part of the placental circulation, a possible explanation for this correlation could be that less vascularized placentas in the first trimester have been exposed to higher pressure in utero and therefore show more pressure increase and less vasodilation *ex-vivo*.

A larger PV at 11 weeks GA was associated with higher baseline pressure in this group of uncomplicated pregnancies. In line with this, previous research by our group showed that baseline pressure during *ex-vivo* perfusion in placentas of pregnancies complicated by early onset PE was significantly lower compared to healthy placentas[21]. These placentas were significantly smaller, exposed to higher blood pressure *in-vivo,* and displayed altered vascular responsiveness. However, the direct response to flow rate increase was not studied. Interestingly, Jones *et al.* did not find the same positive correlation between *in-vivo* umbilical artery Doppler velocimetry and *ex-vivo* feto-placental vascular resistance in placentas of pregnancies complicated by FGR as in healthy placentas[15]. Since smaller first-trimester placental volume is associated with the occurrence of FGR and PE[23], it would be interesting to study whether the correlations seen in the current study also exist in placentas of pregnancies complicated by placental insufficiency (e.g. FGR or PE). Only one patient in the current study delivered a small-for-gestational-age infant, therefore it was not possible to show a clear correlation with fetal growth, however values of these cases were not outliers. Comparing

histology of the included placentas did not provide additional explanations for our results (data not shown). Histological analysis was performed according to the Amsterdam criteria and included maternal stromal-vascular lesions, fetal stromal vascular lesions, infectious inflammatory lesions, immune/idiopathic inflammatory lesions, massive perivillous fibrin(oid) deposition, abnormal placental shape or umbilical insertion site, morbidly adherent placentas (accreta), meconium-associated changes and increased circulating nucleated red blood cells[24].

The differences in findings across the increasing gestational ages could be attributed to the unplugging of the spiral arteries around 9 weeks gestation. In early gestation, cytotrophoblast plugs occlude the spiral arteries, preventing perfusion of the intervillous space to safeguard a low-oxygen environment[25], which is needed for vasculogenesis and cytotrophoblast proliferation[26]. Later in the first trimester, extravillous cytotrophoblast cells invade around the spiral arteries, initiating their remodelling and unplugging[27]. This leads to a low-resistance circulation with an increased perfusion capacity and reduced blood flow velocity into the intervillous space[29,29]. We hypothesize that these vascular modifications impact PV and uPVV measurements and, especially after 9 weeks GA, could result in less pressure increase and more FMVD after delivery for larger PV and uPVV in the late first trimester. We did not demonstrate such an impact for uPVV in this study, but we did observe a negative correlation between PV and pressure increase and a positive correlation between PV and FMVD, in particular after 9 weeks GA.

This study is strengthened by the longitudinal data collection, creating a unique data set combining patient characteristics with *in-vivo* and *ex-vivo* measurements of the placenta. On the other hand, there is a large time gap between our measurements by first-trimester ultrasound and perfusion postpartum. Alterations in placental development during second- and third trimesters could have impacted our results, since capillary growth continues until delivery to accommodate the growing fetus, resulting in an exponential increase in volume of placental vessels in the third trimester[7]. Still, it is known that failure of the maternal spiral arteries to properly remodel in early pregnancy is already associated with higher feto-placental vascular resistance later in pregnancy[15,30]. Despite ongoing alterations, the foundation for placental vascular development is established in the first trimester, and this knowledge supports the correlations found in this study. Further, it remains uncertain whether mode of delivery could have affected vascular resistance. Most placentas in our study were obtained after elective caesarean section (70%) and have not been subjected to labour. Only two placentas were delivered vaginally and one after emergency caesarean section. There is much debate in literature whether mode of delivery affects placental perfusion experiments. On the one hand it has been demonstrated that placentas after vaginal delivery show increased oxidative stress and inflammatory cytokines on both gene- and protein levels[31]. On the other hand, multiple studies showed no difference in placental barrier function during *ex-vivo* perfusion for delivery mode[32,33]. Lastly, identified correlations should be cautiously interpreted due to the small sample size of the study, which also hampered correction for multiple testing. Furthermore, such small sample size could lead to bias. However, values of patients with characteristics that stood out from the rest (e.g. IVF pregnancy, periconceptional smoking, spontaneous delivery), were not outliers. Also, male/female differences could introduce bias. Unfortunately only 3 female neonates were included in this study which made verification of bias impossible, although they were not outliers. A larger sample size would have probably

strengthened the identified correlations, but since this was an explorative study and *ex-vivo* placental perfusion is difficult, expanding the group size within a reasonable time frame was not feasible.

In conclusion, we showed that *in-vivo* larger first-trimester PV and uPVV are associated with less pressure increase and higher FMVD of the *ex-vivo* feto-placental vasculature at term, suggesting that enhanced adaptive mechanisms after delivery relate to a more optimal development of the placenta early in pregnancy. First-trimester evaluation of placental volume and vascularization could therefore be of value to predict placental function in later pregnancy, thereby providing future opportunities for early prevention as well as treatment of pregnancy-related pregnancy complications. As a next step towards this, future research should focus on validation of these measurements in the general population and in placentas from complicated pregnancies (FGR and/or PE).

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CHAPTER 7

General discussion

As a temporary but most unique organ shared by the mother and her unborn child, the placenta has a major impact on maternal and pregnancy outcome and as such also contributes to a solid foundation for new life. Despite its undebatable importance, amazingly, knowledge about placental health remains limited and undervalued in early pregnancy, i.e., the first trimester. The main reason for this is that in clinical practice placenta-related complications present in the second half of pregnancy, when placental development has already been completed. Consequently, opportunities for interventions to improve placental health and to prevent placenta-related complications have already expired.
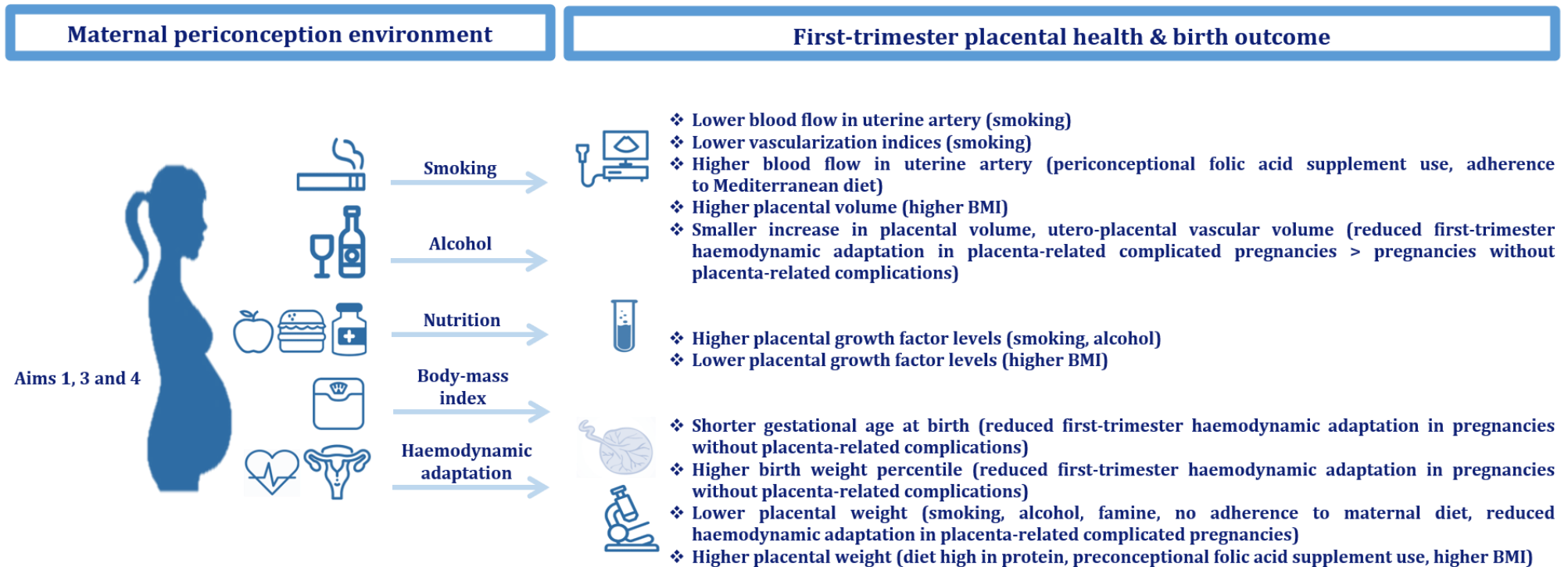
Although knowledge on early placental health is increasing, the tools for early and non-invasive assessment are not widely available. This thesis describes the combination of 3D ultrasound and Virtual Reality as a new method to assess non-invasive *in vivo* markers of placental health. These novel markers are used to investigate associations with periconceptional maternal conditions and lifestyle. Moreover, this thesis describes associations between these markers of placental health and embryonic and fetal growth and birth outcome, and maternal haemodynamic adaptation to pregnancy **(Figure 1 and 2)**.

Placental health in the periconception period: knowledge gaps (Aim 1)

This thesis focuses on early pregnancy and more specific the periconception period, defined as 14 weeks prior to conception up to 10 weeks thereafter, which is unique in the field of placental research[1]. Since the preconceptional state of endometrial receptivity and the process of first-trimester placentation take place in this period, this is the window of opportunity to contribute to placental health. In early gestation, endovascular trophoblastic plugs occlude the spiral arteries, preventing perfusion of the intervillous space to safeguard a low oxygen environment[2] and facilitating vasculogenesis and cytotrophoblast proliferation[3]. Later in the first trimester, extravillous cytotrophoblast cells invade around the spiral arteries, initiating their remodelling and unplugging[4]. This leads to a low-resistance circulation which consists of an increased perfusion capacity and reduced blood flow velocity into the intervillous space to optimize placental development and function throughout pregnancy[5,6]. Derangements in this precarious process can result in aberrant placental development giving rise to placenta-related complications in the second half of pregnancy.

Periconceptional maternal nutrition and lifestyle have been strongly associated with embryonic growth trajectories[7,8]. We hypothesize that these conditions also interfere with early placental development. Therefore, we have evaluated the available literature on the impact of periconceptional maternal smoking, alcohol use, nutrition and body weight on both clinical features and biomarkers of placental development and function throughout pregnancy (Chapter 2). Evidence of the impact of periconceptional maternal lifestyle and nutrition was retrieved, mainly for periconceptional smoking and placental weight at birth as outcome. It is questionable whether placental weight is the most ideal marker for evaluation of placental development and function. Methods to assess placental weight differ and the time gap between the first trimester and birth is large. Other maternal exposures and conditions in between can have an impact as well. Also, an increase or decrease in placental size alone is not always a

Figure 1. Aims and main findings of this thesis (Chapters 2, 5 and 6)

Maternal periconception environment

First-trimester placental health & birth outcome

Smoking

Alcohol

Nutrition

Body-mass index

Haemodynamic adaptation

Aims 1, 3 and 4

- ❖ Lower blood flow in uterine artery (smoking)
- ❖ Lower vascularization indices (smoking)
- ❖ Higher blood flow in uterine artery (periconceptional folic acid supplement use, adherence to Mediterranean diet)
- ❖ Higher placental volume (higher BMI)
- ❖ Smaller increase in placental volume, utero-placental vascular volume (reduced first-trimester haemodynamic adaptation in placenta-related complicated pregnancies > pregnancies without placenta-related complications)

- ❖ Higher placental growth factor levels (smoking, alcohol)
- ❖ Lower placental growth factor levels (higher BMI)

- ❖ Shorter gestational age at birth (reduced first-trimester haemodynamic adaptation in pregnancies without placenta-related complications)
- ❖ Higher birth weight percentile (reduced first-trimester haemodynamic adaptation in pregnancies without placenta-related complications)
- ❖ Lower placental weight (smoking, alcohol, famine, no adherence to maternal diet, reduced haemodynamic adaptation in placenta-related complicated pregnancies)
- ❖ Higher placental weight (diet high in protein, preconceptional folic acid supplement use, higher BMI)

proxy for better or worse placental function. Moreover, a cautious interpretation of the effect estimates is needed due to poorly defined exposures and time windows of investigation, unstandardized measurements of the outcome and small sample sizes of the included studies.

Methodological considerations

Study design

The studies described in this thesis were performed in the Virtual Placenta study, as a sub-cohort of the Rotterdam Periconception Cohort which is a prospective observational cohort study. First-trimester placental measurements were performed longitudinally to assess early placental development in association with embryonic growth, fetal growth and outcome at birth using 3D power Doppler (PD) ultrasound. This is a safe method to assess uterine, embryonic and placental structures during the periconception period and the ALARA-principle was followed at all times[9-13]. The non-invasive and safe character of 3D PD ultrasound with minimal burden for patients is promising for use in future research settings. However, since causality cannot be demonstrated from this observational cohort, our findings should be considered with caution.

Not all participants were included at 7 weeks GA, which explains some missing ultrasound volumes. The highest number of ultrasound volumes was available at 9 weeks GA, since quality loss was more often present at 11 weeks GA due to volume incompleteness or embryonic/fetal movements. In line with the physiology of the exponential embryonic growth velocity, we expected a comparable increase of placental volume and utero-placental vascular volume after 9 weeks of gestation due to unplugging of the spiral arteries. Due to the limited serial samples hampering statistical modelling we were not able to confirm this.

Study population

Inclusion of participants from as early as 7 weeks gestation is unique in the field of Doppler ultrasound, and although challenging, this was feasible in our tertiary hospital because of the focus of our research on the periconception period. The internal validity of the results is high because of this tertiary setting, although this limits the external validity and warrants validation in a general population. Inherent to the tertiary setting, our cohort comprised a large proportion of IVF/ICSI pregnancies, nulliparous women and women with heterogeneous co-morbidities. Moreover, selection bias could have impacted the results since also these participants were more often highly educated, used folic acid supplements and were mainly of Dutch origin. To reduce these biases we adjusted for characteristics that are known to impact placental health[14].

Data accuracy

The addition of VR to assess the uterine, placental and embryonic vasculature enables offline evaluation following ultrasound acquisition and fully benefits the third dimension. This results in more reliable and detailed semi-automated quantification of vascular volumes. At the Erasmus MC there is broad experience of the use of VR to assess placental, embryonic and fetal parameters[15-19]. Despite the recent improvements, performance of semi-automated placental

assessments in VR was limited so far. Novel technical methodology towards fully-automated VR measurements is advocated to facilitate clinical applicability. Therefore, the next step is to develop a method for fully-automated measurement of the uPVV. This seems to be possible on the condition that strictly defined areas within the utero-placental volume can be detected automatically.

Since uPVV represents the maternal part of the placental vascular bed, another aim would be to distinguish between maternal and fetal vasculature in a 3D PD volume using VR. To achieve this, the V-scope software could be extended with advanced 3D morphological algorithms for automated image processing. This enables derivation of quantitative information about utero-trophoblastic vascular characteristics in detail (e.g., vessel size (diameter) and length, number of branches, and blood flow patterns). Also the anatomical visualization and evaluation in VR of the maternal vascular branches itself besides the spiral arteries, such as the arcuate and radial arteries, needs to be unraveled. The addition of machine and deep learning to VR and all other available data will likely aid to achieve these aims in the future[20].

A considered extension of the study design was to visualize the placenta in the second and third trimester using VR. Due to increasing placental size however it was not feasible to record the placenta in total and merging techniques were not applicable for 3D utero-placental volumes at this stage. Improvement of merging techniques for multiple 3D volumes would be of great value to enable assessment of placental health throughout pregnancy. Another improvement would be the use of VR instead of the VOCAL tool to calculate PV. In this thesis, PV was measured using VOCAL software since automated detection of the utero-placental border by VR was not feasible.

Ultrasounds were performed by experienced ultrasonographers, following a clearly defined protocol with use of standardized settings to ensure comparability of the measurements. Despite the use of a preset, power Doppler ultrasound remains sensitive to artefacts during image acquisition and the used preset may not have been appropriate for all patients in the cohort. For example, studies recommend the use of the sub-noise gain to guarantee acquisition of a meaningful image[21]. Future research designed to construct clear guidelines on uniformity in 3D PD ultrasound settings could provide more generable recommendations for clinical settings.

New methods to assess placental health (Aim 1)

In our pilot study, we demonstrate that it is feasible and reliable to visualize the utero-placental and embryonic vasculature in detail from 7 weeks onwards using 3D PD ultrasound and VR (Chapter 3). This shows the applicability of these imaging techniques for assessment of the utero-placental circulation in the early stages of placental development in a longitudinal setting throughout the first trimester.

Figure 2. Aims and main findings of this thesis (Chapters 4 and 6)
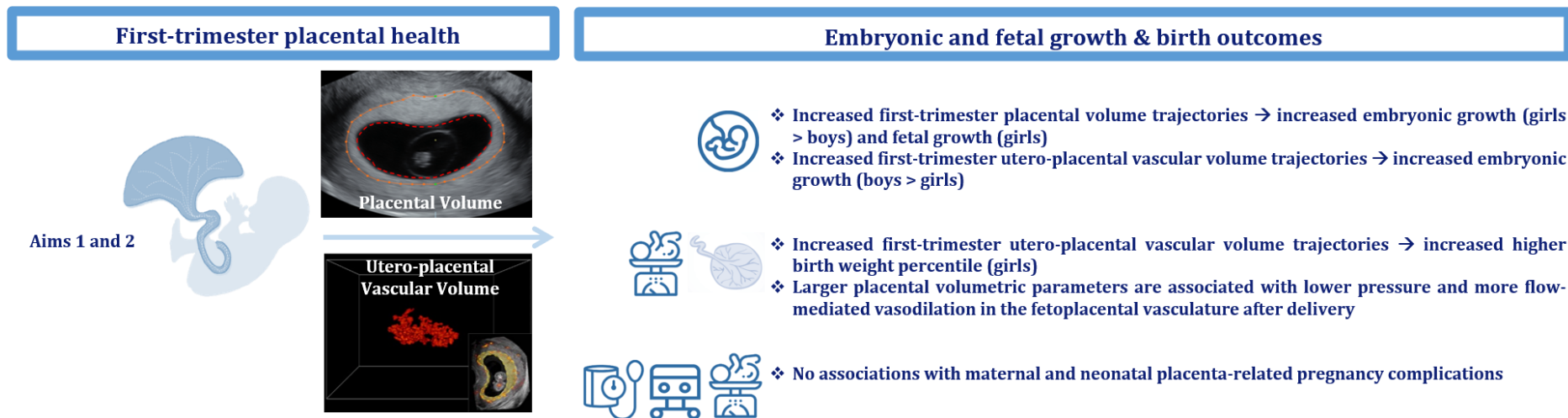
Placental health and perinatal health (Aim 2)

In the Virtual Placenta Study, placental parameters were assessed longitudinally in the first trimester of pregnancy. Since this approach to early placental health is new, this challenged us to define reference curves of PV and uPVV in the early first trimester (Chapter 4). First-trimester placental development was associated with embryonic growth, fetal growth and outcome at birth, with different effect estimates for boys and girls. PV development was associated with an increase in embryonic and fetal growth, in particular in girls. uPVV development was associated with embryonic growth, only in boys, and with birth weight centile only in girls.

Our results suggest that increased first-trimester placental development enhances embryonic and fetal growth and birth weight. Still a cautious interpretation is needed, since we acknowledge that increased trophoblast and placental vascular volume may not be the only characteristic to reflect placental development or function. Parameters for trophoblast tissue metabolism and vascular function will have additional value to represent placental function[22]. Furthermore, a larger increase in uPVV compared to PV in early gestation (thus an increased ratio) could reflect a too early onset of the fetal-maternal circulation by prompt unplugging of the spiral arteries[5], giving rise to excessive oxidative stress. We did not find evidence for the existence of such a phenomenon in our cohort. The exponential increase in volume of placental vessels in the third trimester, resulting from capillary growth that continues until delivery to accommodate the increasing fetal size, can explain why no associations were identified for the outcomes later in pregnancy[23]. In addition, the associations in this cohort were impacted by fetal gender. Male fetuses are of larger size in the first trimester already[24,25]. Also male and female gene expression for placental development is different. Boys direct more energy to body growth and development while girls direct more energy towards placental development[26]. Still, the pathways and origins of the influential factors that cause differences between fetal gender remain largely unclear and should be addressed in future work.

First-trimester placental development was most strongly associated with first-trimester embryonic development, whereas associations with later fetal development and birth outcomes were weaker. Not only does the period between 7 and 11 weeks GA cover exceptionally rapid placental and embryonic development, first-trimester parameters are also temporally remote from second- and third-trimester parameters. In this time frame other factors could interact with the later stages of fetal growth.

It is striking that almost 25% of the women in the Virtual Placenta Study population developed a placenta-related complication. Although this is a relatively high number, which is not surprising in this tertiary population, we identified stronger associations in women with placenta-related complicated pregnancies. The specialized medical care that this tertiary population received may have prevented and treated the (early) onset of these complications. Existing evidence has demonstrated that placenta-related complications such as PE have heterogeneous etiologies with comparable clinical presentations. Early-onset PE is more often associated with abnormal placentation. On the other hand, late-onset PE has been associated with maternal metabolic and cardiovascular risk factors such as obesity and diabetes mellitus rather than abnormal placentation, generating higher susceptibility to vasoactive factors as a result of inflammatory responses[27]. Most complications were of late onset in the Virtual

Placenta Study cohort, which may have altered the effect estimates. Because the study was not powered to evaluate placental development in placenta-related complications, we were not able to investigate this any further.

It was revealed that larger placental volumetric parameters in the first trimester were associated with lower pressure and more dilation of the fetoplacental vasculature after delivery in a subset of 10 placentas (Chapter 6). Although the explorative nature of this study in uncomplicated pregnancies demands further validation of these measurements in placentas from complicated pregnancies, this thesis is the first to demonstrate such associations. In the future, the preventive value of the evaluation of first-trimester placental volume and vascular volume may be considered in the prediction of placental function later in pregnancy. Assessment of correlations with placental histopathological characteristics postpartum may be of value to assess the preventative value of first-trimester placental volume and vascularization.

Historically, the placenta has been studied mainly in the field of gynaecology and obstetrics. The impact that placenta-related complications have on fetal and neonatal health however should raise a discussion on the poor interest of paediatricians for the placenta. The placenta may provide key opportunities for early interventions to improve fetal and subsequent neonatal health. The benefit from these opportunities and collaborations between disciplines will eventually result in better birth outcomes and enhanced cost-effectiveness due to less and shorter neonatal intensive care unit admissions.

The impact of maternal conditions on placental health (Aim 3)

Reduced first-trimester maternal haemodynamic adaptation, reflected by higher first-trimester MAP and UtA blood flow resistance was associated with decreased placental development, i.e., PV and uPVV. At birth, reduced first-trimester maternal haemodynamic adaptation to pregnancy was associated with lower placental weight in placenta-related complicated pregnancies, when reflected by resistance to UtA blood flow. Pregnancies with placenta-related complications demonstrated that an increase in MAP trajectories was associated with a higher birth weight centile. Moreover, an increased resistance to UtA blood flow was associated with a shorter GA at birth (Chapter 5). Although median values for MAP and UtA blood flow were still within the normal clinical ranges in women developing placenta-related complications, their median values were already higher at baseline. The established cut-off values may not succeed to reveal women at risk for aberrant placental development and function in the early stages of pregnancy. To improve early detection we recommend to re-evaluate alternative methods to clinically assess haemodynamic adaptation reflected by MAP and UtA blood flow. Assessment of MAP and UtA trajectories preconceptional and in the first trimester rather than single measurements at baseline may be a more sensitive approach. Our results warrant investigation in a general study population to evaluate the attributive value of VR placental parameters to assess first-trimester maternal haemodynamic adaptation.

Our findings are in line with the theory that pregnancy acts as a stress test for the maternal cardiovascular system, which suggests that mothers with impaired haemodynamic adaptive capacity are at higher risk to develop placenta-related complications[28-30]. However, it is difficult to determine whether less increase in uPVV is an actual result of maternal vascular impairment

or whether smaller uPVV lead to higher UtA resistance due to a reactive response. More evidence is suggesting that changes in maternal utero-placental blood flow as reflected by UtA Doppler indices do not directly result from trophoblast invasion, but that maternal haemodynamics prior to pregnancy determine placental development[26,31,32]. Assessment of periconceptional determinants of maternal haemodynamics in the future, could unravel causative pathways in order to ameliorate preconceptional screening and care to prevent placenta-related complications in pregnancy[33].

A well-studied maternal characteristic in association with placental development is parity. It is known that placentas of nulliparous women are on average smaller at birth than those of multiparous women[34-36] and that nulliparous women more often develop placenta-related complications[37]. A similar difference between nulliparous and multiparous women was expected for first-trimester placental development. Although placental volumes of nulliparous women were larger at 7 weeks GA, but grew slower than the placentas of multiparous women later in the first trimester, associations were not different for parity in the Virtual Placenta Study (data not shown). An explanation may be the heterogeneity of the indications for tertiary care within this population or the influence of other cohort characteristics such as age, educational level or lifestyle on these associations. Other studies report that structural changes induced by pregnancy do not obliterate completely after birth and that spiral artery wall compliance and distension persist following trophoblast invasion[38]. As a result, more effective remodeling of spiral arteries occurs in future pregnancies. This provides an explanatory pathway to the diminished risk for placenta-related complications in parous women. Moreover, more extensive trophoblastic invasion of the decidual vessels in parous women has been described[39]. An animal study investigating consequences of nitric oxide deficiency in the first pregnancy, which may hamper physiologic vascular adaptation in pregnancy, observed a more physiological vascular adaptation in the second pregnancy[40]. Recently, it was reported that the initial course of early placental vascular development is different in multiparous women when expressed in ultrasound placental vascularization indices and blood biomarkers[34].

The impact of the preconception period and conception mode (Aim 1)

A very interesting, but so far largely neglected topic is the contribution of the preconceptional endometrium to optimal placentation. Since the endometrium is crucial to placentation, future research should address the impact of maternal conditions and lifestyle on the quality of the preconceptional endometrium[41,42]. Although nowadays it is more feasible to recruit women preconceptionally, in particular subfertile women, it remains more challenging to recruit women from the general population[43]. Besides, it is a challenge to investigate the endometrial vasculature in spontaneous cycles at similar time points as well as in both spontaneous and stimulated cycles, to estimate the window of implantation. Moreover, the duration of the implantation window may vary with several days, i.e. diapause, which influences the interpretation of placental and fetal growth[44].

Previously, it has been demonstrated that assisted reproduction alters embryonic growth trajectories, likely due to hormonal stimulation and different conditions of the in vitro culture that cause altered embryonic gene expression. Not only does this affect fetal growth and

development, but this can be translated into altered placental development[45,46]. The risk for pregnancy complications like FGR and PE are elevated after assisted reproductive techniques[47-49]. It is hypothesized that also the maternal vascular system is influenced by ovarian stimulation in IVF/ICSI treatment which may create less optimal endometrial conditions for placental development due to the process of endometrial decidualization that is endocrine-driven in assisted reproduction[50]. Again this is in line with the stress test theory of pregnancy.

We were not able to confirm an unfavorable environment for placental development in IVF/ICSI pregnancies in this thesis. In fact, women who conceived after IVF/ICSI treatment had similar PV and uPVV and slightly lower UtA indices compared to women who conceived spontaneously (data not shown). The previously reported increased risk for pregnancy complications may result from factors that are not related to placental development or manifestation of placental dysfunction after the first trimester of pregnancy.

Future implications for research and clinical practice

The ultimate objective of this thesis was to enhance awareness on the importance of a healthy lifestyle for placental health. Since we show that maternal periconceptional lifestyle impacts placental development, this supports further development of lifestyle interventions and strategies to benefit placentation. However, a different approach to the assessment of early placental health in clinical practice requires future validation of our findings in a general population. First, to disclose underlying mechanisms of the associations between early placental development and pregnancy course and outcome. Second, to focus on the influence of periconceptional maternal haemodynamic adaptation and lifestyle on placental development and subsequent pregnancy outcomes. Programs to promote folic acid supplement use, smoking cessation and optimizing diet prior to and during pregnancy are already available. In particular personal mHealth coaching programs (www.slimmerzwanger.nl and smarterpregnancy.co.uk) are very much appreciated[51-53]. All couples contemplating pregnancy should be offered a face-to-face preconceptional consultation through health care centers to assess risk factors in lifestyle and nutrition and provide them with affordable evidence-based tools such as the mHealth program Smarter Pregnancy to improve their lifestyle and nutritional behavior[54-58].

More insight into the maternal exposome in association with early placental health could further contribute to create risk profiles to identify mothers at risk for adverse placental outcomes. The non-genetic human exposome has been described to complement the human genome, expressed in a lifelong general and specific external environment (including lifestyle factors)[59]. Factors within the exposome require further elaboration in future research.

Last but not least, we propose a move towards more investigation into the paternal role in placentation. Besides a maternal origin, the placenta is also a largely paternally-expressed organ. Previous studies have suggested a role of the father in the etiology of PE. The limited exposure to paternal components prior to conception has been mainly proposed as a mechanism. This is underlined by the higher prevalence of PE in nulliparous women, multiparous women with changing partners, a long interval between pregnancies and ICSI-conceived pregnancies[60]. Genetically, the risk of PE can be attributed to the mother for 35%,

fetus for 20% and couple for 13%. More knowledge on this topic is needed as part of a multifactorial approach to placental health.

General conclusion

With this thesis we highlighted the relevance of the periconception period to investigate placental health. A shift towards early assessment of placental development is where we can gain most to prevent placenta-related complications throughout pregnancy. Our results show that first-trimester PV and uPVV measured by 3D ultrasound and VR could be promising non-invasive markers to identify adverse placental development. It was demonstrated that first-trimester placental development is not uniform and aberrant development can already be linked to altered embryonic growth, but also fetal growth, fetoplacental vasculature at term and birth weight centiles, with modification by fetal gender. Moreover, decreased first-trimester maternal haemodynamic adaptation has a negative impact on first-trimester placental development, in particular in placenta-related complicated pregnancies.

In due time, placental parameters measured by VR could be part of a strategy for accurate prediction of placenta-related complications. Ultimately, they could contribute to the selection of patients who might benefit from preventive and therapeutic modalities for placenta-related complications, starting as early as the periconception period.

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CHAPTER 8

Summary (English)
Samenvatting (Dutch)

English summary

The placenta forms the connection between the haemodynamic system of mother and child (fetus). The course of pregnancy is therefore inextricably linked to the placenta. The placenta plays a role in maternal adaptations to pregnancy and is responsible for the supply of nutrients to the child and the removal of wastage products. Prior to pregnancy, the endometrium prepares for implantation of the embryo after fertilization and at the same time, placental development starts as well. A low-oxygen environment is required in the first 10 weeks of pregnancy for optimal placental development. This low-oxygen state is guaranteed by small placental tissue plugs within the maternal spiral arteries that restrain blood flow from mother to child. Around the 9th week of pregnancy, these plugs dissolve and blood flow between mother and child through the placenta starts, which is followed by a widening of the spiral arteries to ensure adequate supply of nutrients and oxygen from mother to child. Placental development is impacted by derangements in the process of spiral artery remodeling. If the placenta is not developed optimally and functions suboptimally, short-term pregnancy complications can arise as a result, such as high blood pressure and preeclampsia in the mother and growth restriction of the fetus. But there are also long-term consequences for the health of mother and child. In addition, a history of placenta-related complications gives a higher risk of recurrence for poor placental development and function in subsequent pregnancies.

The period from 14 weeks before conception up to 10 weeks thereafter is important for the early development of the placenta, since the endometrium already prepares for embryonic implantation in the weeks prior to pregnancy. This period is also being referred to as the periconception period. During this period maternal conditions such as age, parity and also nutrition and lifestyle can influence placental development. In daily practice there is no optimal method available to assess placental development in early pregnancy. Various methods for the assessment of placental development and function have been investigated, such as determining substances (i.e. biomarkers) in the blood that are released by the placenta. Not only such biomarkers can reflect placental development, histopathological examination of the placental tissue can also be performed following miscarriage or delivery. By using ultrasound, an imaging technique commonly used in pregnancy, the placenta itself, but also the blood flow to and within the placenta can be visualized during pregnancy. By recording and saving images of the placenta, with and without imaging of blood flow, the size of the placenta and the amount of vasculature can be displayed lifelike and measured using Virtual Reality techniques. This offers an opportunity to assess the placenta early and non-invasively and to study associations with maternal nutrition and lifestyle, embryonic and fetal growth and birth outcomes.

A systematic review is described in **Chapter 2**, in which we address the existing knowledge on the influence of maternal lifestyle factors in the periconception period on the development and function of the placenta during pregnancy. In particular, evidence of an adverse effect of smoking was demonstrated on the development and function of the placenta. The design and methods of the studies retrieved were limited, so the results must be interpreted with caution. Future research should focus more on the physiological consequences of an unhealthy lifestyle during the periconception period. Moreover, we anticipate that new evidence will further

support the development of lifestyle interventions to improve the health of mothers and their offspring from the earliest moment in life.

In **Chapter 3** we describe the development of a feasible and reliable method to assess the vascular volume of the uterus and placenta (utero-placental vascular volumes) prior to conception and in the first trimester of pregnancy. A new method was developed since the availability of imaging makers of early placental development is still limited. The placental measurements were performed using three-dimensional power Doppler (3D PD) ultrasound volumes on two different Virtual Reality (VR) systems, to use the third dimension in the image optimally. We showed that vascular volume measurements of the uterus, the embryo and the placenta using 3D PD ultrasound and VR are feasible and reliable. Repeated measurement studies are needed to further validate this and to assess their value as new imaging markers for placental development and ultimately for the prediction of placenta-related complications.

In a follow-up study, 3D PD ultrasound is combined with the Virtual Organ Computer-aided Analyzis (VOCAL)™ tool and VR for longitudinal assessment of placental health in the early first trimester, as described in **Chapter 4.** These measurements are performed in the Virtual Placenta Study, embedded in the Rotterdam Periconception Cohort (Predict Study). In this chapter we study the associations between utero-placental (vascular) development in the first trimester and embryonic growth, the growth of the fetus in the second and third trimester and birth outcomes. A total of 214 pregnant women underwent serial three-dimensional (3D) ultrasound scans at 7, 9 and 11 weeks gestational age (GA) to obtain volumes that covered the whole gestational sac. First-trimester placental development was associated with embryonic growth, fetal growth and outcome at birth, with different results for boys and girls. Placental volume development was associated with an increase in embryonic and fetal growth, in particular in girls. Development of utero-placental vascular volumes was associated with embryonic growth, only in boys, and with birth weight centile only in girls. Concluding, early measurements of placental development using 3D ultrasound and VR in the future could contribute to the early assessment of and interventions in embryonic, fetal and neonatal health.

Previous studies suggest that maternal haemodynamic adaptation is associated with the risk to develop placenta-related complications. In **Chapter 5**, it is investigated if first-trimester maternal haemodynamic adaptation, reflected by the course of alterations in first-trimester mean arterial pressure and impedance to uterine artery blood flow, is associated with first-trimester placental development and birth outcomes. Reduced first-trimester maternal haemodynamic adaptation, reflected by higher first-trimester mean arterial pressure and uterine artery blood flow resistance, was associated with decreased first-trimester placental development (placental volume and utero-placental vascular volume). At birth, reduced first-trimester maternal haemodynamic adaptation to pregnancy, reflected by higher resistance to uterine artery blood flow, was associated with lower placental weight in in placenta-related complicated pregnancies. Pregnancies with placenta-related complications showed an association between an increase in mean arterial pressure and higher birth weight centile and increased resistance to uterine artery blood flow and a shorter gestational age at birth. These findings suggest that the development of placental tissue and vasculature are affected

differently in pregnancies with or without placenta-related complications. Moreover, the impact of first-trimester haemodynamic (mal)adaptation on placental development seems more profound in placenta-related complicated pregnancies.

In **Chapter 6** we describe the relationship between measurements of placental (vascular) volumes using 3D ultrasound and VR in the first trimester, and measurements of blood flow in the fetal part of the placenta after birth, in a sub-cohort of 10 placentas included in the Virtual Placenta Study that were collected postpartum. Immediately after delivery, blood flow in the placenta was artificially restarted to measure pressure in the vessels located in the fetal side of the placenta. The pressure in the fetal placental vessels was gradually increased, to be able to measure how the vessels in the placenta react to the increase in pressure. In a normally functioning placenta, the vessels should relax as the pressure increases, so that optimal blood flow in the placenta is maintained. We found that there was an association between larger placental (vascular) volumes in the first trimester and lower pressure and more dilation after delivery in the fetal part of the placenta. This suggests that larger and/or more vascularized placentas in early pregnancy have better adaptive mechanisms to pressure increase and possibly lead to better pregnancy outcomes.

The concluding **Chapter 7** summarizes the findings from this thesis and provides recommendations for the future, not only for research settings addressing early placental health, but also for clinical opportunities to assess early placental health.

Nederlandse samenvatting

De moederkoek, ook wel de placenta, vormt de verbinding tussen de bloedstroom van moeder en kind (foetus). Het beloop van de zwangerschap is hierdoor onlosmakelijk verbonden met de aanleg en ontwikkeling van de placenta. De placenta speelt ook een rol bij aanpassingen van het lichaam van moeder aan de zwangerschap en is verantwoordelijk voor de aanvoer van voedingstoffen naar het kind en de afvoer van afvalstoffen. Al vóór de zwangerschap wordt het baarmoederslijmvlies (endometrium) voorbereid op de innesteling van het embryo na de bevruchting. Tegelijkertijd met de bevruchting start ook de ontwikkeling van de placenta. Voor een goede placentaontwikkeling is in de eerste 10 weken van de zwangerschap een zuurstofarme omgeving nodig. Deze (zuurstofarme) omgeving wordt gewaarborgd door kleine weefselpluggen in de spiraalarteriën van het moederlijke deel van het placentabed, die de bloedstroom van moeder naar het kind blokkeren. Vanaf ongeveer 9 weken zwangerschap verdwijnen deze pluggen en komt de bloedstroom op gang tussen de circulatie van de moeder en de foetus via de placenta. Na het verdwijnen van de pluggen zorgt de verwijding van de spiraal arteriën voor voldoende aanvoer van benodigde voedingstoffen en zuurstof van moeder naar kind. Als deze processen niet optimaal verlopen heeft dit effect op de ontwikkeling van de placenta. Wanneer de placenta niet optimaal is aangelegd en functioneert, kunnen als gevolg hiervan zwangerschapscomplicaties ontstaan, zogenaamde placenta-gerelateerde zwangerschapscomplicaties. Deze korte termijn complicaties zijn onder meer hoge bloeddruk en zwangerschapsvergiftiging (preeclampsie) bij de moeder en groeivertraging van het kind. Maar er kunnen ook gevolgen zijn voor de gezondheid van moeder en kind op de lange termijn. Bovendien geeft het doormaken van zulke placenta-gerelateerde zwangerschapscomplicaties een hoger herhalingsrisico van deze complicaties in volgende zwangerschap(pen).

Omdat het endometrium zich al voorafgaand aan de zwangerschap voorbereid op de innesteling van het embryo, is de periode van 14 weken voor de bevruchting tot 10 weken daarna ook belangrijk voor de vroege placentaontwikkeling. Deze periode wordt ook wel de periconceptieperiode genoemd. In deze periode kunnen kenmerken van de moeder zoals leeftijd, pariteit maar ook voeding en leefstijl de placentaontwikkeling beïnvloeden. In de dagelijkse praktijk is er nog geen optimale methode beschikbaar om al zo vroeg in de zwangerschap de placentaontwikkeling te beoordelen. Eerder zijn er verschillende methoden onderzocht, zoals het bepalen van markers is het bloed (biomarkers), dit kunnen producten zijn die afkomstig zijn uit biologische processen. Deze biomarkers komen vervolgens vrij vanuit de placenta in het bloed van de moeder. Biomarkers kunnen daarom gebruikt worden als een afspiegeling van de placenta ontwikkeling, maar ook histopathologisch onderzoek van placentaweefsel na een miskraam of geboorte geeft informatie over de ontwikkeling van de placenta. Met behulp van echoscopie, hetgeen veel gebruikt wordt als beeldvormende techniek in de zwangerschap, kan de placenta zelf, maar ook de bloedstroom naar en in de placenta afgebeeld worden. Door de placenta af te beelden met driedimensionale echoscopie en deze beelden op te slaan, met en zonder weergave van de bloedstroom, kunnen de placentagrootte en de hoeveelheid bloedvaten hierin levensecht getoond en gemeten worden met behulp van Virtuele Realiteit (VR). Dit biedt de kans om al op vroege en niet-ingrijpende wijze de placenta

te kunnen beoordelen en vervolgens te kijken naar de verbanden met moederlijke voeding en leefstijl, de embryonale en foetale groei en geboorte-uitkomsten.

In **Hoofdstuk 2** is in een systematische review de bestaande kennis beschreven over de invloed van moederlijke leefstijl factoren in de periconceptieperiode op de ontwikkeling en functie van de placenta gedurende de zwangerschap. Met name werd er bewijs gevonden voor een nadelige invloed van roken op de ontwikkeling en functie van de placenta. De opzet en methoden van de gevonden onderzoeken waren wisselend van kwaliteit waardoor de resultaten voorzichtig geïnterpreteerd moeten worden. Toekomstig onderzoek zou zich meer moeten richten op de gevolgen van een ongezonde leefstijl op de placentaontwikkeling tijdens de periconceptieperiode. Bovendien voorzien we dat nieuw bewijs de ontwikkeling van leefstijlinterventies verder zal stimuleren om de gezondheid van moeders en hun nakomelingen vanaf het vroegste moment in het leven te verbeteren.

In **Hoofdstuk 3** beschrijven we de verdere ontwikkeling van een haalbare en betrouwbare methode om reeds vóór de bevruchting en in het eerste trimester van de zwangerschap het bloedvatvolume te beoordelen van de baarmoeder en placenta (utero-placentaire vaatvolumes). Met behulp van driedimensionale power Doppler (3D PD) echoscopie werd de placenta afgebeeld en gemeten door middel van twee verschillende VR systemen, zodat optimaal gebruik wordt gemaakt van diepte als derde dimensie. We toonden aan dat vaatvolume metingen van de baarmoeder, het embryo en de placenta met behulp van 3D PD echoscopie en VR haalbaar en betrouwbaar zijn. Onderzoek met metingen op meerdere momenten in een zwangerschap is nodig om dit verder te bevestigen en de waarde te beoordelen als nieuwe beeldvormende methode voor de placentaontwikkeling en uiteindelijk voor de voorspelling van placenta-gerelateerde zwangerschapscomplicaties.

Het onderzoek met herhaalde metingen van de placenta in de eerste drie maanden van de zwangerschap met behulp van 3D PD echoscopie beschrijven we in **Hoofdstuk 4**. Deze metingen zijn verricht in de Virtual Placenta Studie, als onderdeel van het Rotterdam Periconceptie cohort (Predict Studie). In dit hoofdstuk bestuderen we de associaties tussen de ontwikkeling van de placenta(bloedvat) volumes in het eerste trimester en de embryonale groei, de ontwikkeling van de foetus in het tweede en derde trimester en uiteindelijke geboorte-uitkomsten. Een totaal van 214 zwangere vrouwen onderging seriële 3D echo's bij 7, 9 en 11 weken zwangerschapsduur waarmee 3D volumes zijn verkregen die de hele zwangerschap omvatten. Eerste trimester placentaontwikkeling was geassocieerd met embryonale groei, foetale groei en geboorte-uitkomst, met verschillende resultaten voor jongens en meisjes. Toename van placentavolume was geassocieerd met een grotere embryonale en foetale groei, die meer uitgesproken was bij meisjes. Bij jongens werd een positieve associatie aangetoond tussen de toename van placentavaatvolume en de embryonale groei. Bij meisjes was er bij een toename van het placentavaatvolume sprake van een hoger geboortegewichtpercentiel. Concluderend kunnen vroege metingen van placentaontwikkeling met behulp van 3D echoscopie en VR in de toekomst mogelijk bijdragen aan de vroege diagnostiek naar embryonale, foetale en neonatale gezondheid en de mogelijkheid om vroegtijdig te behandelen.

Eerdere studies suggereren dat moederlijke hemodynamische aanpassing, oftewel aanpassing van het moederlijke hart- en vaatstelsel in de zwangerschap, verband houdt met het risico om placenta-gerelateerde zwangerschapscomplicaties te ontwikkelen. In **Hoofdstuk 5** is onderzocht of de aanpassingen in het moederlijke hemodynamische systeem in het eerste trimester, afgeleid uit de veranderingen in de moederlijke bloeddruk en de bloedstroom in de slagader van de baarmoeder (arteria uterina) geassocieerd zijn met de placentaontwikkeling in het eerste trimester, de groei van de foetus en de uitkomst van de zwangerschap. Verminderde moederlijke hemodynamische aanpassing aan de zwangerschap in het eerste trimester, weergegeven door een hogere gemiddelde bloeddruk en weerstand in de slagaders van de baarmoeder, ging gepaard met afgenomen placenta-ontwikkeling (een kleinere toename van placentavolume en placentavaatvolume). Zwangerschappen die gepaard gingen met placenta-gerelateerde complicaties vertoonden een associatie tussen verminderde hemodynamische aanpassing, weergegeven door een hogere weerstand in de slagaders van de baarmoeder, en een lager placentagewicht en kortere zwangerschapsduur bij de geboorte. Wanneer verminderde hemodynamische aanpassing, weergegeven werd door een hogere gemiddelde bloeddruk, ging dit gepaard met een hoger geboortegewichtpercentiel. Onze bevindingen suggereren dat de ontwikkeling van het placentaweefsel en het placentavaatstelsel anders wordt beïnvloed in zwangerschappen met of zonder placenta-gerelateerde complicaties. Bovendien lijkt de impact van (verminderde) hemodynamische aanpassing in het eerste trimester op de placentaontwikkeling groter bij zwangerschappen met placenta-gerelateerde complicaties.

In **Hoofdstuk 6** beschrijven we de relatie tussen de metingen van placenta (bloedvat)volumes met behulp van 3D echoscopie en VR in het eerste trimester, en metingen van de bloedstroom in het foetale gedeelte van de placenta na de geboorte, in 10 van de placenta's die verzameld zijn na de bevalling in de Virtual Placenta studie deelnemers. Direct na de bevalling werd de bloedstroom in de placenta opnieuw kunstmatig op gang gebracht om zo de druk te meten in de vaten die gelegen zijn in het foetale gedeelte. De druk in de foetale vaten van de placenta werd geleidelijk opgevoerd om te bepalen hoe deze reageren op druktoename. In een normaal functionerende placenta horen de vaten te ontspannen wanneer de druk toeneemt, zodat de bloedstroom in de placenta zo optimaal mogelijk is. Er bleek een associatie te zijn tussen grotere placenta (vaat)volumes in het eerste trimester en een lagere druk en meer verwijding van de vaten in het foetale gedeelte van de placenta na de bevalling. Deze bevinding kan erop wijzen dat grotere en/of placenta's met meer vaten in de vroege zwangerschap zich beter kunnen aanpassen aan druktoename en daarmee mogelijk leiden tot betere zwangerschapsuitkomsten.

Het afsluitende **Hoofdstuk 7** vat de bevindingen uit dit proefschrift samen en geeft aanbevelingen voor de toekomst, zowel voor het verrichten van onderzoek naar de vroege placentaire gezondheid als op het vlak van de klinische implementatie van de beoordeling van vroege placentaire gezondheid.

ADDENDUM

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List of abbreviations
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LIST OF ABBREVIATIONS

1-C	One carbon
2D	Two-dimensional
3D	Three-dimensional
AFP	Alpha-fetoprotein
ALARA	As Low As Reasonably Achievable
ART	Assisted reproductive techniques
BAX	Bclr-like protein 4
BCL2	Protein encoded by BAX gene
BDNF	Brain derived neurotropic factor
β-hCG	Beta human chorionic gonadotropin
BMI	Body-mass index
CD36	Cluster of differentiation 36
CI	Confidence interval
cIAP	Cellular inhibitors of apoptosis
cm	Centimeters
cm^2	Squared centimeters
cm^3	Cubic centimeters
CRL	Crown-rump length
CV	Coefficient of variation
EFW	Estimated fetal weight
EV	Embryonic volume
FABP	Fatty acid translocase binding protein
fβhCG	fetal beta human chorionic gonadotropin
FI	Flow index
FGFR	Fibroblast growth factor
FGR	Fetal growth restriction
FMVD	Flow-mediated vasodilation
GA	Gestational age
GH	Growth hormone
hCG	Human chorionic gonadotropin
hPL	Human placental lactogen
ICC	Intraclass correlation coefficient
ICSI	Intracytoplasmic sperm injection
IGF-1	Insulin-like growth factor-1
IQR	Interquartile range
IU	International units
IUGR	Intrauterine growth restriction
IUI	Intrauterine insemination
IVF	In vitro fertilization
kg	Kilograms
LMP	Last menstrual period
LPL	Lipoprotein lipase
m	Meters
MAPK	Mitogen-activated protein kinase
MCA	Middle cerebral artery
miRNA	Micro ribonucleic acid
μg	Micrograms
mg	Milligrams
mL	Milliliters
mm	Millimeters

mm²	Squared millimeters
MoM	Multiples of the median
mRN	Messenger ribonucleic acid
NF-κB	Nuclear factor kappa B
ng	Nanogram
OR	Odds ratio
PAI	Plasminogen activator inhibitor
PAPP-A	Pregnancy-associated plasma protein A
PD	Power Doppler
PE	Preeclampsia
pg	Picogram
PI	Pulsatility Index
PlGF	Placental Growth Factor
PIH	Pregnancy-induced hypertension
PSV	Peak systolic velocity
PTB	Preterm Birth
PUFA	Polyunsaturated fatty acids
PV	Placental volume
PVV	Placental vascular volume
PWR	Placental weight ratio
uPVV	Utero-Placental vascular volume
RI	Resistance Index
SLC27A4	Subtype of fatty acid translocase
SD	Standard deviation
sEng	Soluble endoglin
SGA	Small-for-gestational age
sFlt-1	Soluble Fms-like tyrosine kinase 1
SOD	Superoxide dismutase
TauT	Taurine transporter protein
TP53	Tumor protein p53
TRWB	Tropomyosin receptor kinase B
TSPO	Mitochondrial translocator protein
TVV	Total vascular volume
UK	United Kingdom
U/L	Units per liter
UmbA	Umbilical Artery
USA	United States of America
UtA	Uterine Artery
UVV	Uterine Vascular Volume
VEGF	Vascular Endothelial Growth Factor
VFI	Vascularization-flow index
VI	Vascularization index
VOCAL	Virtual Organ Computer-aided AnaLysis
VR	Virtual Reality

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This thesis
<u>Reijnders IF</u>, Mulders AGMGJ, Koster MPH. **Placental development and function in women with a history of placenta-related complications: a systematic review.** Acta Obstet Gynecol Scand. 2018;97(3):248-257.

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e-BROK course and certificate (NFU BROK Academy)	2016	1.0
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Biostatistics for clinicians part I (NIHES)	2016	1.4
Principles of epidemiologic data analysis (NIHES)	2016	1.4
Systematic literature retrieval course in Pubmed (Erasmus MC)	2015	0.3
EndNote course (Erasmus MC)	2015	0.1
Other attended seminars, conferences and courses		
Monthly academic center of excellence, pregnancy and child, meetings	2018-2019	0.5
Biomedical English writing and communication	2018-2019	3.0
16th National course of pediatric pathology, practical placental pathology	2017	0.3
Masterclass and certification Fetal Anomaly Ultrasound Scan, Erasmus MC	2016-2017	2.0
Erasmus MC PhD day	2016	0.2
Centre for Trophoblast Research Annual Trophoblast Meeting, Cambridge, UK	2016	1.0
Annual Wladimiroff award meeting, department of obstetrics and gynaecology	2015-2018	0.5
Annual Sophia Research Day meeting, Erasmus MC	2015-2018	0.5
Three-monthly meeting Rotterdamse Gynaecologen Opleidings Cluster (RGOC)	2015-2019	0.5
Weekly research meeting of the department of obstetrics and gynaecology (& presentations)	2015-2019	1.0
Presentations at (inter)national conferences		
57th Gynaecongres, annual meeting of the Dutch Society for Obstetrics and Gynaecology, oral presentation	2020	1.0
67th annual meeting of the Society for Reproductive investigation, Vancouver, poster presentations (preparations only)	2020	1.0
30th World Congress on Ultrasound in Obstetrics and Gynecology, ISUOG, Berlin, Germany, poster presentation (preparations only)	2019	0.3
11th Developmental Origins of Health and Disease, Melbourne, Australia, oral presentation (preparations only)	2019	1.0

11th Developmental Origins of Health and Disease, Melbourne, Australia, poster presentation (oral presentation and poster preparations only)	2019	0.6
Academic center of excellence meeting, pregnancy and child, oral presentation	2019	0.3
10th Developmental Origins of Health and Disease, Rotterdam, oral presentation	2017	1.0
28th World Congress on Ultrasound in Obstetrics and Gynecology, ISUOG, Vienna, oral presentation	2017	0.3
64th annual meeting of the Society for Reproductive investigation, Orlando, poster presentation	2017	1.0
Sophia Research Day, Erasmus MC, oral presentation, awarded for best pitch	2017	0.3
Academic center of excellence meeting, pregnancy and child, oral presentation	2017	0.3
Erasmus MC Bridge meeting, oral presentation	2017	0.3
Research meeting of the department of reproductive medicine, oral presentation	2017	0.3
Research meeting of the department of obstetrics and gynaecology, oral presentations	2015-2019	0.3

Teaching experience

Supervision Master Thesis Annelieke Kropman	2018-2019	2.0
Tutor of Medical students, bachelor year 1	2018-2019	1.0
Minor mystery of creation: placental measurements in Virtual Reality	2017	0.3
Supervision Melissa van der Windt, extracurricular	2016-2017	1.0
Supervision Master Thesis Kalijn Rijnders	2016	2.0
Practical education placental examination, Medical students, bachelor year 3	2015-2018	0.5

ABOUT THE AUTHOR

Igna Reijnders was born in 1989 in Eindhoven, the Netherlands as the eldest of five siblings. She grew up in Wijchen for most part of her childhood, were she attended middle school. Her enthusiasm for obstetrics was already triggered by witnessing the pregnancy of her youngest sister and brother. An obstetric case during introduction classes at the Radboud University in Nijmegen convinced her to study Medicine. She enrolled in 2007 and conducted a research internship at the Princess Anne maternity Hospital, Southampton, United Kingdom in 2011 to study placental size in relation to birth weight, supervised by prof. dr. N. Macklon and prof. D. Braat. Little did she know that this topic would relate to her future PhD thesis.

Back in the Netherlands, her enthusiasm for research was wide awake and motived her to evaluate Anti-Müllerian Hormone levels in association with live birth in women undergoing assisted reproduction under the supervision of prof. dr. D. Braat. After graduation in 2014, her wish to become a gynaecologist led her to Delft to gain experience a residency in obstetrics/gynaecology in the Reinier the Graaf hospital under the supervision of dr. H.A. Bremer. In 2015 she started studying towards her PhD degree under the supervision of prof. dr. R.P.M. Steegers Theunissen to focus on the assessment of early placental health using three-dimensional ultrasound and virtual reality.

In 2020 Igna started as a resident in obstetrics and gynaecology at the Reinier de Graaf Gasthuis in Delft, under the supervision of dr. K. Kapiteijn en dr. B.S.M. Verbruggen.

Igna is married to Christiaan Kwint and together they have two sons, Victor and Boris.

DANKWOORD

Een promotietraject is een tijd van persoonlijke groei, van vallen en opstaan. Met name is het een tijd van doorzetten. Graag spreek ik mijn dank uit aan hen zonder wie dit nooit was gelukt.

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