Periconceptional maternal and paternal homocysteine levels and early utero-placental (vascular) growth trajectories: The Rotterdam periconception cohort

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ABSTRACT

Introduction: Maternal elevated plasma total homocysteine (tHcy) is involved in the origin of several placenta-related pregnancy complications. The first trimester is the most sensitive period for placentation influenced by maternal and paternal health. The aim is to study associations between periconceptional parental tHcy levels and utero-placental growth trajectories in the first trimester of pregnancy.

Methods: Pregnant women and their partners were enrolled before 10 weeks of gestation in the Virtual Placenta study as subcohort of the Rotterdam periconception cohort (Predict study). A total of 190 women with a singleton pregnancy, of which 109 conceived naturally and 81 after IVF/ICSI treatment, were included. We measured serial utero-placental vascular volumes (uPVV) and placental volumes (PV) at 7, 9 and 11 weeks of gestation. First-trimester trajectories of PV were also measured in 662 pregnancies from the total Predict study.

Results: Comparing all participants of the virtual placenta study, no association between maternal tHcy and uPVV was observed. However, in IVF/ICSI pregnancies sub-analyses showed significantly negative associations between maternal tHcy in the 3rd and 4th quartile and uPVV trajectories (beta: -0.38 (95%CI -0.74 to -0.02) and beta: -0.42 (95% CI -0.78 to -0.05), respectively) with the 1st quartile as reference. Analysis in the total Predict cohort showed similar negative associations for the total study population.

Discussion: Periconceptional high maternal tHcy levels are associated with smaller placental growth trajectories depicted as PV and uPVV in the first trimester of pregnancy. The stronger negative associations with uPVV in IVF/ICSI pregnancies underline the need for further investigation.

1. Introduction

The placenta plays a crucial role in embryonic growth and development by facilitating gas-exchange of oxygen and carbon dioxide, and the supply of important nutrients, such as glucose, amino acids and calcium [1]. Following conception, placentation starts with endometrial invasion by the embryonic trophoblast cells, followed by the formation and remodeling of maternal utero-placental vessels [2]. Impaired early placentation is associated with placenta-related (vascular) pregnancy complications later in gestation, such as miscarriage, fetal growth restriction, and (early-onset) preeclampsia with high morbidity and mortality in both mother and child [3,4].

Homocysteine, a key component in one-carbon (1-C) metabolism, plays a role in early placentation involving cellular metabolism and proliferation, as well as in the regulation of gene expression through epigenetic mechanisms [5]. Plasma total homocysteine (tHcy), a key element for protein- and (phospho)lipid synthesis, is converted into methionine using folate and vitamin B12 catalyzed by methionine synthase. Methionine is converted to S-adenosylmethionine (SAM) serving as main 1-C donor for methylation of several biological substrates, such as DNA and RNA.
as proteins, lipids and RNA, DNA involved in epigenetic processes [5].
Subsequently, low levels of B-vitamins, are associated with a mild to moderate increase of tHcy. tHcy is also involved in the scavenging of reactive oxygen species (ROS), after trans sulphuration into cysteine and cysteine. Cysteine is bounded to glutamate for synthesis of the antioxidant glutathione. Therefore, elevated tHcy may serve as biomarker of excessive oxidative stress. Excessive oxidative stress is strongly linked to endothelial dysfunction and thrombosis ultimately causing cardiovascular disease [6,7]. Preconception maternal hyper-homocysteinemia is associated with placenta-related (vascular) pregnancy complications, yet the impact on early placental development is not known [8,9].

In men, plasma tHcy affects sperm parameters and hyper-homocysteinemia is associated with increased DNA damage to sperm, which is relevant since paternally imprinted genes are predominantly expressed in the placenta [10,11]. Also, alterations in 1-C metabolism in men have been shown to affect embryonic development and growth [12]. Since plasma tHcy is strongly associated with placenta-related pregnancy complications in women and with sperm parameters in men, we hypothesize that elevated periconceptional parental tHcy is negatively associated with early-pregnancy utero-placental (vascular) development. Therefore, the aim of this study is to investigate associations between both periconceptional maternal and paternal plasma tHcy levels and early utero-placental (vascular) development in order to further optimize placental vascular development in the future.

2. Materials and methods

2.1. Ethical approval

This study was approved by the Medical Ethical and Institutional Review Board (MEC 2015–494) of the Erasmus MC, University Medical Centre, Rotterdam, the Netherlands. Both women and men provided written informed consent at enrolment.

2.2. Study design

Between January 2017 until March 2018 participants were enrolled in the Virtual Placenta Study (registration number Dutch Trial Register: NTR6854), a subcohort of the Rotterdam Periconception Cohort (Predict study), which focuses on placental imaging from the first trimester of pregnancy onwards [13]. Since 2016 the virtual Placenta study, embedded in the Rotterdam periconception cohort (Predict study) is designed for in-depth investigation of placental development using ultrasound imaging, performing measurements of the utero-placental vascular volume (uPVV). These detailed vascular volumes were not part of and collected in the original Predict study. The Predict Study is an ongoing prospective cohort study, performed at the Department of Obstetrics and Gynecology at the Erasmus MC, University Medical Center Rotterdam. The Netherlands [13]. Inclusion criteria for both women and their partners were as follows: at least 18 years of age, a singleton pregnancy and the ability to speak and read the Dutch language. Women and their partners were included before the 10th week of gestation, with pregnancies conceived either naturally or after In Vitro Fertilization (IVF) with or without Intracytoplasmic Sperm Injection (ICSI) treatment.

2.3. Study parameters

After enrollment participants filled out a general questionnaire, addressing topics like geographical background, education, smoking, alcohol and use of vitamins. At the moment of inclusion, height, weight and blood pressure of the couples were measured and a non-fasted blood sample was taken. Blood samples were collected in Vacutainer ethylenediaminetetraacetic acid (EDTA) tubes. Centrifugation of the EDTA-plasma took place within 10 min to prevent an artificial increase of tHcy [14].

After centrifugation, determination of tHcy was performed with a Waters Quattro Premier liquid chromatography-tandem mass spectrometry system (Waters, Milford, Massachusetts, United States). The interassay coefficient of variation was <5.5%.

For naturally conceived pregnancies, gestational age (GA) was determined by the first day of the last menstrual period (LMP). For participants who had a regular menstrual cycle of less than 25 days or more than 31 days, GA was adjusted for duration of the menstrual cycle. When GA determined by LMP and measurement of the crown rump length (CRL) indicated a difference of more than 7 days, or when LMP was missing, GA was estimated by the CRL at the 9 weeks ultrasound scan.

When pregnancies were conceived after IVF/ICSI, GA was calculated by adding 14 days to the date of oocyte pick-up for IVF with or without ICSI. If a cryopreserved embryo was used, GA was calculated by adding 19 days to the transfer day, since thawed cryopreserved embryos were transferred at day 5 of embryo development.

2.4. Ultrasound and outcome variables

Placental volume (PV, cm³) was measured in both the Virtual Placenta and Predict study using 3D ultrasound volumes of the whole pregnancy obtained at 7, 9 and 11 weeks of gestation, using a 6–12 MHz transvaginal probe compatible with the GE Voluson E8 Expert System. We measured PV offline, using Virtual Organ Computer-aided Analysis (VOCAL), which was proven to be a valid technique to measure PV in the first trimester of pregnancy (intraclass correlation coefficients (ICC) > 0.97) [15]. To assess PV, 12 sections of the trophoblast were made with a rotational step of 15°. Subsequently the total pregnancy volume and the gestational sac in each section were measured manually and PV was calculated by subtracting the gestational sac from the total pregnancy volume (Fig. 1A) [15].

Utero-Placental Vascular Volume (uPVV, cm³) was measured exclusively in the Virtual Placenta Study. In short, to measure uPVV, 3D Power Doppler (PD) ultrasound volumes of the pregnancy were obtained at 7, 9 and 11 weeks of gestation using a 6–12 MHz transvaginal probe compatible with the GE Voluson E8 Expert System. 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 ‘low 1’, quality ‘high’) [16,17]. Furthermore constant default instrument settings were used throughout the examinations with the following settings: frequency, low; dynamic, set 5; balance, 180; smooth, 4/5; ensemble, 12; line density, 8; power Doppler map, 5; artifact suppression, on; power Doppler line filter, 1; quality, high. Assessment of the uPVV in VR, using a semi-automated technique offline, enabled accurate measurement by projecting the volume as a real-life image, i.e. ‘a hologram’, which could be rotated and enlarged for more precision [16]. This procedure proved to be a valid technique (intraclass correlation coefficients ICC between 0.94 and 0.97). At first, all embryonic structures were removed from the hologram and secondly the myometrium was removed, leaving only the vessels up to the utero-placental border, and thus the uPVV (Fig. 1B). After measurement of both uPVV and PV, we calculated the ratio of those parameters, which was used to determine the amount of vasculature per cm³ of placental volume.

2.5. Statistical analysis

Pregnancies were excluded from analysis after oocyte donation, miscarriage or congenital abnormality. Participants without available tHcy or placental measurements were also excluded.

First, we analyzed maternal and paternal baseline characteristics of the total study population, in natural conceptions and in IVF/ICSI pregnancies. We compared the distribution of baseline variables
between IVF/ICSI and naturally conceived pregnancies using the medians and interquartile ranges for continuous variables and percentages for categorical variables. We tested for differences using the Mann-Whitney U test or the Chi-squared test, respectively. To assess associations between longitudinal measurements of PV, uPVV and uPVV/PV with tHcy of both women and men, we used linear mixed models. For analysis of the trajectories of PV, uPVV and uPVV/PV ratio, we used a third root transformation of these variables to establish linearity between these parameters and GA.

To further investigate associations between tHcy and PV, uPVV and uPVV/PV ratio, we divided tHcy levels of the Virtual Placenta study participants into quartiles (Q) and used Q1 as reference category. Maternal quartiles were as follows: Q1: 3.80–5.39 μmol/L; Q2: 5.40–6.29 μmol/L; Q3: 6.30–7.29 μmol/L; and Q4: 7.30–53.70 μmol/L.

For the linear mixed models analyzing the association between maternal tHcy and PV, uPVV, uPVV/PV ratio, we first adjusted only for GA (Model 1). Subsequently, in model 2 we additionally adjusted for the maternal covariates age, body mass index (BMI), alcohol use, ethnicity, education and parity, which were selected based on literature and baseline differences. When analyzing associations between paternal tHcy and PV, uPVV, uPVV/PV ratio, we additionally adjusted for the paternal covariates age, alcohol use and education. Furthermore, we adjusted for paternal tHcy when analyzing associations of maternal tHcy and vice versa and additionally adjusted for GA at blood withdrawal. P-values <0.05 were considered statistically significant. All analyses were performed using SPSS package 24.0 (IBM SPSS Statistics, Armonk, NY).

2.6. PV measurements in the predict study

We analyzed the associations between parental periconceptional tHcy and PV trajectories in the total cohort of the Predict study. We evaluated associations between maternal and paternal tHcy and PV using linear mixed models with the same models and confounders of the subcohort. Associations with the different maternal tHcy quartiles were analyzed using the cut-off points from the subcohort since these were nearly similar (Supplemental Table 1).

2.7. Role of the funding source

The funding source had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

3. Results

3.1. Virtual placenta study (uPVV, PV)

In this subcohort, we included 241 participants. Prior to analysis, we excluded participants because of oocyte donation (n = 3), miscarriage (n = 22), congenital malformation (n = 9) and drop-out (n = 1). Furthermore, we excluded women without available blood samples (n = 8) or placental measurements (n = 8), leaving a total of 190 women for analysis, of which 109 pregnancies were naturally conceived and 81 pregnancies were conceived after IVF/ICSI. We included a total of 146 men, of which 81 pregnancies were naturally conceived and 65 pregnancies were conceived after IVF/ICSI (Fig. 2).

At baseline, only a significant difference in the number of nulliparous women between the natural pregnancy group and the IVF/ICSI group (44% vs 77.8% respectively (p < 0.001)) was noted (Table 1). The median tHcy in the first trimester of pregnancy was 6.3 μmol/L (IQR 5.4–7.3 μmol/L) for the total group, 6.4 μmol/L (IQR 5.4–7.3 μmol/L) for the naturally conceived pregnancy group and 6.1 μmol/L (IQR 5.5–7.0 μmol/L) for the IVF/ICSI group (p = 0.24).

The regression analyses of women of the total study population and
the naturally conceived pregnancy group showed no statistically significant associations between tHcy and PV, uPVV or uPVV/PV ratio. In the subgroup of IVF/ICSI pregnancies, there was a significantly negative association between tHcy and uPVV, and uPVV/PV ratio (beta 0.132 (95% CI: 0.235 to 0.029) and beta 0.032 (95% CI: 0.064 to 0.001) respectively) after adjustment for the confounders specified before (model 2) (Table 2). The regression analyses of Model 1 for the IVF/ICSI group showed negative associations regarding uPVV trajectories when comparing Q3 and Q4 with Q1 as reference (Q3 beta: -0.21 (95% CI -0.52 to 0.11), Q4 beta: -0.29 (95% CI -0.61 to 0.03)) (Table 3). After adjusting for confounders these negative associations are significantly different for Q3 and Q4 with Q1 as reference (Q3 beta: -0.42 (95% CI -0.78 to -0.05)). The confounders with the strongest effect were maternal educational level and alcohol use. We found no significant associations between tHcy and uPVV when stratified for fetal sex (Supplemental Table 2).

Retransformation of the effect estimates for uPVV of the IVF/ICSI group to the original scale showed that maternal uPVV in Q4 was reduced by 16.69 cm$^3$ (86.7%) at 11 weeks of gestation compared to the reference group of women with a tHcy in Q1 (Fig. 3). We found no significant differences between tHcy concentrations of women with or without pre-eclampsia (6.4 vs 6.3 μmol/L respectively (p = 0.71)), with or without pregnancy-induced hypertension (7.2 vs 6.3 μmol/L respectively (p = 0.32)) and with or without preterm birth (6.6 vs 6.3 μmol/L respectively (p = 0.16)).

3.2. Men

At baseline there were no significant differences in the study population (Table 1). Median tHcy was 12.2 μmol/L (IQR 10.0–15.2 μmol/L) in the total study population. Regression analysis (Model 2) of the total study population showed no significant associations between paternal tHcy and PV and uPVV (beta: 0.006 (95% CI: 0.018 to 0.007) and beta: 0.011 (95% CI: 0.026 to 0.004)) respectively (Table 2).

3.3. Predict study (PV)

In total 662 women were included, of which 252 conceived after IVF/ICSI and 410 after natural conception. At baseline there were statistically significant differences between naturally conceived pregnancies and pregnancies after IVF/ICSI regarding maternal age, parity, tHcy, BMI and alcohol use, paternal age and vitamin use (Supplemental Table 3). When using maternal Q1 of the subcohort as reference, we found a significantly negative association between maternal tHcy in the total study population (Model 2) and PV for Q3 and Q4 (beta 0.12 (95% CI -0.22 to -0.01) and beta 0.12 (95% CI -0.23 to -0.02) respectively) (Table 4). Regression analysis (model 1) in the IVF/ICSI group showed a significant negative association, when using maternal Q1 as reference, for tHcy in Q3 and Q4 (beta -0.14 (95% CI -0.26 to -0.01) and beta -0.18 (95% CI -0.32 to -0.04) respectively). After correction for confounders this effect attenuated for Q3 and Q4 as compared to Q1 (beta -0.09 (95% CI -0.24 to 0.06) and beta -0.09 (95% CI -0.26 to 0.07) respectively), also showing larger effects in the
Reduced by 10.5 cm

Table 1: Characteristics of the female (n = 147) and male participants (n = 81) stratified for mode of conception.

| Age (years) | naturally conceived pregnancies | IVF/ICSI pregnancies | P-value |
|-------------|---------------------------------|----------------------|---------|
| 31.9        | n = 109                         | n = 10               | 0.084   |
| 31.5        | [29.0-35.3]                     | [28.3-34.5]          | [29.3-36.3] |

Nulliparous

Geographic origin

| Dutch | Western | Non-Western | Educational level | Low | Intermediate | High | At the first trimester study entry: BMI, measured (kg/m²) | Folic acid supplement use | Startled | Use of other vitamins | Alcohol consumption, yes | Smoking | tHcy, plasma, μmol/L | Men |
|-------|---------|-------------|------------------|-----|--------------|------|----------------------------------------------------------|---------------------------|---------|---------------------|------------------------|----------|---------------------|------|
| 147   | 6       | 34          | 1.3              | 13  | 64           | 110  | [22.2-28.0]                                               | 188                        | 0.220   | 0.001*              | 0.806                   | 0.971    | 6.3 [5.4-7.3]      | 0.240 |
| 75.5  | 4.3 (8) | 22.0 (8)    |                  | 7.6 |
| 11.8  | 34 (32.1)| 65 (61.3)   |                  | 48 |
| 97.5  | 25 (25.9)| 65 (61.3)   |                  | 21 |
| 79.7  | 16 (13.8)| 36 (47.4)   |                  | 11 |
| 97 (9.5)| 15 (19.7)| 26 (43.3)    |                  | 16 |
| 49 (89.2)| 20 (19.1)| 26 (43.3)    |                  | 16 |
| 81 (100.0)| 24 (24.1)|              |                  | 24 |

Data are presented as median [interquartile range (IQR)] or n (%). *statistically significantly different between the three groups.

IVF/ICSI group as compared to the total and natural conception population. Retransformation of the effect estimates for PV of the IVF/ICSI group to the original scale showed that PV of maternal tHcy in Q4 compared to Q1 (plasma tHcy >7.3 μmol/L versus <5.40 μmol/L) was strongly associated with reduced PV and uPVV trajectories.

The pathophysiology of hyperhomocysteinemia is very much related to impairment of the vascular system, in particular of endothelial dysfunction, thrombosis and atherosclerosis ultimately leading to coronary artery disease and placental related pregnancy outcomes [21]. As an example, vascular endothelial growth factor (VEGF) is one of the most important growth factors stimulating neovascularization, which is also essential for placental development. Impaired vascularization due to reduced expression levels of VEGF combined with apoptosis, can interfere with placental development, leading to reduced nutrient and gas exchange between the maternal and fetal circulation of placenta-related pregnancy complications. Differences in epigenetic profiling and expression of VEGF can be due to periconceptional exposure to increased tHcy and as such influence the amount and volume of vessels in the developing placenta. This is further substantiated by the associations between hyperhomocysteinemia and decreased concentrations of glutathione causing excessive oxidative stress and apoptosis and dysfunction of the endothelium [22].

The associations shown with uPVV were only present in the IVF/ICSI group, which might be explained by differences in epigenetic programming and associated gene-expression in the placenta related to the IVF/ICSI-procedures [23]. Previous research showed that analysis of genome-wide mRNA expression in placentas of pregnancies conceived after IVF/ICSI compared to naturally conceived pregnancies, show an overexpression of 13 biological pathways, that play an important role in cell cycle control, metabolism, immune response, and 1-C metabolism [23]. Studies investigating women undergoing ovarian stimulation in IVF/ICSI cycles show increased blood vessel density, suggesting advanced endometrial angiogenesis [24]. Hence, the effect estimates of vascular damage associated with high tHcy are larger.

No significant associations between paternal tHcy and uteroplacental (vascular) volumes were found. We hypothesized the effect of paternal tHcy to be large, since growth of the placenta is largely driven by paternal genomic imprinting. Previous studies also showed a fetal sex dependent manner of placental development [17]. Animal studies showed that knockout models of paternaly imprinted genes resulted in a significant reduction of placental size. The exact role of tHcy in this mechanism is not yet understood, however a high concentration of tHcy is deleterious, because it increases excessive oxidative stress and subsequent global and DNA hypomethylation, and efflux of methionine, necessary for normal methylation. The uPVV is greatly influenced by the local maternal environment, indicating that the (epi) genetic paternal influences on the utero-placental vasculature development in early pregnancy are difficult to be detected and distinguished from maternal factors. As a consequence, the paternal influence will initially not outweigh the maternal influence on the first trimester uPVV.

Early placentation is clinically important, since abnormal implantation and early placental development is associated with miscarriages, fetal growth restriction and hypertensive disorders of pregnancy. Although it was not the primary aim of this study, we did not find associations between paternal tHcy and the occurrence of placentula-related vascular complications in pregnancy. As previous research showed associations between maternal tHcy and placenta-related pregnancy complications in cohorts of approximately 8000 participants, most likely our study was not powered to significantly detect differences in uPVV and PV regarding to pregnancy outcomes. Instead, our study provided more insight into the early (patho)physiology of the role of tHcy on uteroplacental (vascular) development [8]. Although the effect estimates also early placental development, such as folate rich dietary patterns and folic acid supplement use.

Previous research demonstrated that elevated plasma tHcy significantly increases the odds for several placenta-related pregnancy complications [8,18,19]. tHcy is associated with placental weight at birth, i.e., tHcy of >8.3 μmol/L is associated with 30 g lower placental weight [20]. These findings are in agreement with our study, where maternal tHcy in Q4 compared to Q1 (plasma tHcy >7.3 μmol/L versus <5.40 μmol/L) was strongly associated with reduced PV and uPVV trajectories.
our study. Through placental volume but also by other mechanisms not measured in our study.

Model 2: Model 1 adjusted for gestational age. Model 2 men: Model 1 + maternal covariates age, parity, BMI, alcohol use, ethnicity, education, tHcy and paternal covariates age, education and alcohol use. Model 2 women: Model 1 + maternal covariates age, parity, BMI, alcohol use, ethnicity, education, tHcy and paternal covariates age, education and alcohol use. Abbreviations: uPVV: utero-Placental Vascular Volume, PV: Placental Volume, CI: Confidence Interval.

Table 2
Subcohort (Virtual Placenta Study): Effect estimates for associations between periconceptional parental tHcy and trajectories of utero-placental (vascular) volumes and stratified for mode of conception.

| tHcy | Model 1 | Model 2 |
|------|---------|---------|
|      | PV (cm³) | uPVV (cm³) | uPVV/PV (µ/L) | PV (cm³) | uPVV (cm³) | uPVV/PV (µ/L) |
| **Total study population** | | | | | | |
| Women n = 190 | | | | | | |
| −0.012 | 0.12 | 0.001 | 0.87 | 0.002 | 0.31 | −0.028 | 0.25 | 0.003 | 0.91 | 0.003 | 0.74 |
| Men n = 146 | | | | | | |
| −0.006 | 0.28 | −0.008 | 0.26 | −0.003 | 0.52 | −0.006 | 0.38 | −0.011 | 0.16 | −0.003 | 0.20 |
| **Naturally conceived pregnancy** | | | | | | |
| Women n = 73 | 0.21 | 0.005 | 0.56 | 0.003 | 0.20 | −0.031 | 0.37 | 0.020 | 0.57 | 0.013 | 0.19 |
| Men n = 59 | −0.026 | 0.23 | −0.012 | 0.002 | 0.008 | −0.098 | 0.037 | −0.052 | 0.092 | −0.007 | 0.032 |
| **IVF/ICSI pregnancy** | | | | | | |
| Women n = 65 | −0.049 | 0.17 | −0.078 | 0.08 | −0.011 | 0.40 | −0.002 | 0.96 | −0.132 | 0.01 | −0.032 | 0.046 |
| Men n = 51 | 0.022 | 0.83 | 0.009 | 0.005 | 0.015 | 0.156 | 0.092 | 0.096 | 0.029 | 0.001 |

Model 3: adjusted for gestational age.

Model 2: Model 1 + maternal covariates age, parity, BMI, alcohol use, ethnicity, education, time of blood withdrawal and paternal covariate tHcy. Model 2 men: Model 1 + maternal covariates age, parity, BMI, alcohol use, ethnicity, education, tHcy and paternal covariates age, education and alcohol use. Abbreviations: uPVV: utero-Placental Vascular Volume, PV: Placental Volume, CI: Confidence Interval.

Table 3
Subcohort (Virtual Placenta Study): Effect estimates for associations between tHcy divided in quartiles (Q) and trajectories of utero-placental (vascular) volumes for the women in the IVF/ICSI population.

| IVF/ICSI pregnancy homocysteine | Model 1 | Model 2 |
|------|---------|---------|
|      | PV (cm³) | uPVV (cm³) | uPVV/PV (µ/L) | PV (cm³) | uPVV (cm³) | uPVV/PV (µ/L) |
| **Q1, 3.8–5.39 µmol/L N = 21** | | | | | | |
| Q2 5.40–6.29 µmol/L N = 20 | | | | | | |
| Q3 6.30–7.29 µmol/L N = 20 | | | | | | |
| Q4 7.30–5.37 µmol/L N = 20 | | | | | | |

Model 1: adjusted for gestational age.

Model 2: Model 1 + maternal covariates age, parity, BMI, alcohol use, ethnicity, education, time of blood withdrawal and paternal covariate tHcy. Abbreviations: uPVV: utero-Placental Vascular Volume, PV: Placental Volume, CI: Confidence Interval, Q: Quartile.

appear to be rather small, retransformation to the original values showed that PV in Q3 and Q4 are 70.9% and 86.7% smaller compared to Q1, which can be clinically relevant, but also asks for further research on the predictive value of PV for adverse placental-related outcome.

Although studies have shown that placental volume is associated with preeclampsia, also other pathophysiological mechanisms are involved such as abnormal spiral artery remodeling and increased placental capillary density [25, 26]. Probably the association between tHcy and placenta related pregnancy outcomes is not only mediated through placental volume but also by other mechanisms not measured in our study.

Plasma tHcy in our study population was rather low (median 6.3 µmol/L (IQR 5.4–7.3 µmol/L)) compared to the cut off value of (<12 µmol/L) for pregnancy, due to the high percentage of folic acid supplement use [27]. In a large population-based cohort mean tHcy levels were 6.9 µmol/L, with the lowest quintile <5.8 µmol/L, which is comparable to our cohort [28]. tHcy concentrations were higher in men than in women. Studies in general populations in both Asia and Europe showed tHcy concentrations averaging between 14.6 and 15.7 µmol/L in men and 9.6 and 13.1 µmol/L in women [29, 30]. Men in our population had a median concentration of 12.2 µmol/L. We need to take into consideration that tHcy concentrations can vary between different ethnic populations, but also differences in nutrition, internal metabolic parameters and age. Moreover, men in our study were recruited from a tertiary care center, also including men with underlying diseases and medication use, that can interfere with homocysteine metabolism.
Concentrations of tHcy tend to decrease later in pregnancy, which is most likely caused by hemodilution. In our study, participants were included and blood was taken between week 7 and 9 of gestation and to overcome problems with differences in tHcy based on hemodilution, we also corrected for gestational age at the moment of blood withdrawal. We found the effect of timing of blood withdrawal, however, to be very small, without large impact on our study results. We used non-fasted blood samples from participants for logistical reasons. Moreover, there is short-term biological variation of tHcy after meals and the necessity for fasting has been questioned because of similar fasting and non-fasting tHcy reference values [31,32]). Future research should investigate the optimal ranges for maternal tHcy with regard to adverse placenta-related pregnancy outcomes.

The main strengths of our study are the longitudinal early measurements of PV and uPVV using standardized 3D ultrasound combined with VR. These measurements were assessed by experienced researchers and these techniques are proven to be valid with excellent reproducibility [16]. As a strength we used a predefined preset of power Doppler settings. However, using the same gain for all participants may not be appropriate, since individual patient characteristics may need different ultrasound settings to overcome ultrasound artefacts. A recent study proposed using individualized sub-noise settings to obtain images with minimalized artefacts [33]. Furthermore we are aware of the applicability of the placental parameters VI, FI and VFI for evaluation of the placental vasculature [34]. The ultrasound parameters VI, FI and VFI are derived from 3D ultrasound data, like uPVV and PV. Performing these VI, FI and VFI measurements, despite using 3D ultrasound data, only 2 planes (i.e. a sagittal and transversal), are used to calculate and describe the placental parameters. In our VIRTUAL study, the availability of 3D datasets and offline VR analysis provides the opportunity to perform volumetric measurements using 3D data most optimally. In addition to 3D ultrasound datasets VR actually enables real depth perception. Hence, 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). Another strength of our study is the measurement of tHcy in the first trimester of pregnancy in both women and men. Moreover, we also measured PV in the Rotterdam Periconception cohort with over 600 participants. Limitations of our study are the relatively small sample size (n = 190) for investigating the associations with uPVV. Furthermore, our study population was recruited from a tertiary hospital, which largely comprises high-risk pregnancies, complicating the extrapolation of our results to a general population. The current study is performed for hypothesis generating, inherent to this aim and design, we accepted higher type I errors and have chosen not to correct for multiple comparisons using statistical procedures such as Bonferroni corrections.

5. Conclusions

In this manuscript we provide a (patho)physiological insight into the associations between in particular maternal tHcy and early utero-placental (vascular) development. As a unique finding, we demonstrate that high periconceptional maternal tHcy, especially in pregnancies after IVF-ICSI, is associated with decreased utero-placental (vascular) volumes, in a dose-dependent way with the potential to
reduce maternal and neonatal complications after reaching the viable stage of pregnancy, which needs further investigation.

Authors' roles
RST initiated the research question and supervised all aspects of the study. JH and IR contributed to data acquisition. SW and RST initiated and supervised the statistical procedures of the manuscript. JH, BR SS wrote the first draft. All authors interpreted the data and all authors contributed to the writing and the critical revisions of the manuscript and all authors approved the final version of the manuscript and authorized the submitted version.

Data sharing
Data is available upon request.

Declarations of competing interest
None.

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Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2021.09.012.

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