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## Cohort Profile

# Cohort Profile Update: the Rotterdam Periconceptual Cohort and embryonic and fetal measurements using 3D ultrasound and virtual reality techniques

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## The original cohort

For more than three decades, extensive translational research has identified strong associations between fetal growth and development and fetal programming of health and diseases in later life [Developmental Origins of Health and Disease (DOHAD) hypothesis].<sup>1,2</sup> More recently, the focus of DOHAD research has shifted to the periconceptual period, defined as the time window of 14 weeks before up to 10 weeks after conception, thereby covering the vulnerable processes of gametogenesis, embryogenesis and initiation of placentation. During this period, numerous molecular and biological processes are initiated, such as epigenetic modification (e.g. genome-wide methylation) and also unique transcriptional and translational activities. The periconceptual period as such represents a critical and vulnerable time window for a diversity of exposures with potentially large effects during the entire prenatal as well as postnatal life course. So far significant associations have been detected between maternal and paternal

periconceptual exposures, pregnancy outcome and non-communicable diseases over the entire life course and in later life.<sup>3–6</sup>

The Rotterdam Periconceptual Cohort (Predict study) was initiated because most birth cohorts start enrolment and data collection during the second half of pregnancy or at birth, thereby ignoring the periconceptual window and the first half of pregnancy.<sup>7</sup> Within this cohort we have designed serial patient consultations, including ultrasound measurements starting in the embryonic period. Morphological parameters as well as early placentation are being studied using innovative virtual reality imaging techniques. The Predict study is designed as a tertiary hospital-based, prospective open birth cohort study, with a focus on three research areas: (i) determinants of maternal and paternal periconceptual health; (ii) reproductive performance, pregnancy course and outcome; and (iii) underlying molecular biological mechanisms, such as 1-carbon metabolism and epigenetics and also cardiovascular and

### Key Features

- The Rotterdam Periconceptual Cohort study was set up in 2009 to investigate maternal and paternal periconceptual health and the impact on reproduction, pregnancy and neonatal outcome.
- In the pilot phase 233 pregnancies were included (2009–10) and in the study phase 2717 pregnancies were included (2010–18), participants being aged between 19 and 48.
- What is new in the cohort:
  - Novel three-dimensional (3D) ultrasound and virtual reality measurements and their protocols are introduced, such as the first-trimester head volume and uterine vascular placental volume. The measurements of the inner organ systems, e.g. the kidneys and lungs, are studies under development.
  - A new focus is the fully automated analyses of 3D ultrasound data, resulting in a 3D ultrasound embryonic and fetal brain atlas by using artificial intelligence.
  - Ongoing unique subcohorts comprise: (i) the Virtual Placenta study aiming to establish periconceptual and (patho)physiological maternal cardiovascular and uterine vascular placental mechanisms contributing to the origin of placenta-related pregnancy outcome; (ii) the Virtual Pre-implantation Embryo study focusing on (pre-implantation) embryonic growth and development; and (iii) the HAPPO study, focusing on maternal haemodynamic parameters during the preconception period and pregnancy.
  - The introduction of the mHealth online lifestyle coaching platform, Smarter Pregnancy, at the outpatient clinic and linkage to the administrative database of the Erasmus MC allow access of all health care personnel to personalized coaching advice.
- To study the impact of above-mentioned associations, a nested randomized controlled trial will start in 2021. The trial is designed as a survey and will use a periconceptual blended lifestyle care approach.
- The Predict study can be contacted for collaborations: [predictstudie@erasmusmc.nl].

inflammatory mechanisms. The strength of this open and ongoing cohort lies in the fact that the various outcomes within these three research areas are of equal significance for the periconceptual period. Hypothesis-driven specific outcome parameters are defined before initiation of new studies within the cohort. Only after approval of a new study proposal can the particular study be initiated within the cohort. The achieved output very much depends on the topic of (inter)national scientific research calls, and thus funding.

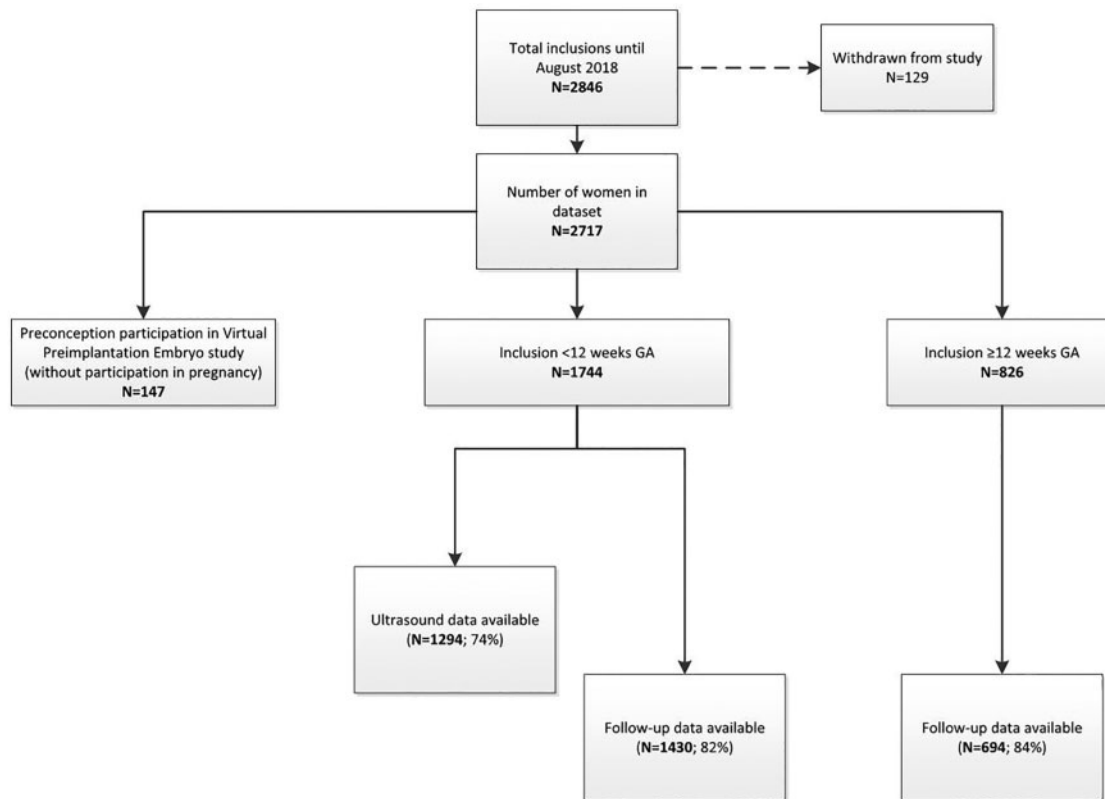
The study started in November 2010 (after a pilot phase initiated in December 2009) and its ongoing cohort design serves as an infrastructure for embedding other subcohort studies, investigating research questions and measuring additive outcome parameters in more detail.<sup>7</sup>

All participants are recruited from the outpatient clinic of the Erasmus MC, University Medical Center, a Dutch tertiary referral centre for predominantly high risk pregnancies. Inclusion criteria are: (i) women (and their partner) should at least have reached the age of 18; (ii) proficiency in speaking and reading the Dutch language. Annually, about 2500 couples visit the Erasmus MC outpatient clinic for preconception or pregnancy consultation. The tertiary setting allows for a yearly capacity of first-trimester serial ultrasound measurements in at least 250 pregnancies.

In total, 2950 couples have been included in the period between December 2009 and August 2018; 233 pregnancies from the preconceptional period up to 8 weeks of gestation were enrolled during the pilot phase (December 2009 and November 2010). A total of 2717 couples participated in this cohort between November 2010 and August 2018. Of these couples, 1891 women were included during the periconceptional period and 826 pregnancies ranging from 13 weeks of gestation until term. For the current profile update paper, it was decided to include all results until August 2018, since all participants had delivered by that time.

During the pilot phase, women underwent weekly 3D transvaginal ultrasound examinations between 6 + 0 and 12 + 6 weeks of gestational age and inclusion was required <8 weeks of gestation (Figure 1). Serial investigation during the first trimester included two-dimensional (2D) and 3D transvaginal ultrasound examinations at 7, 9 and 11 weeks of gestational age. The subcohorts of the Dream and Virtual Placenta study included additional 2D and 3D transabdominal ultrasound examinations at 22, 26 and 32 weeks of gestation (Figure 2).

A more detailed description of the cohort is provided by the cohort profile article of Steegers-Theunissen *et al.*<sup>7</sup> In short, women, and their partners, who are scheduled for a first prenatal visit at the outpatient clinic, are actively



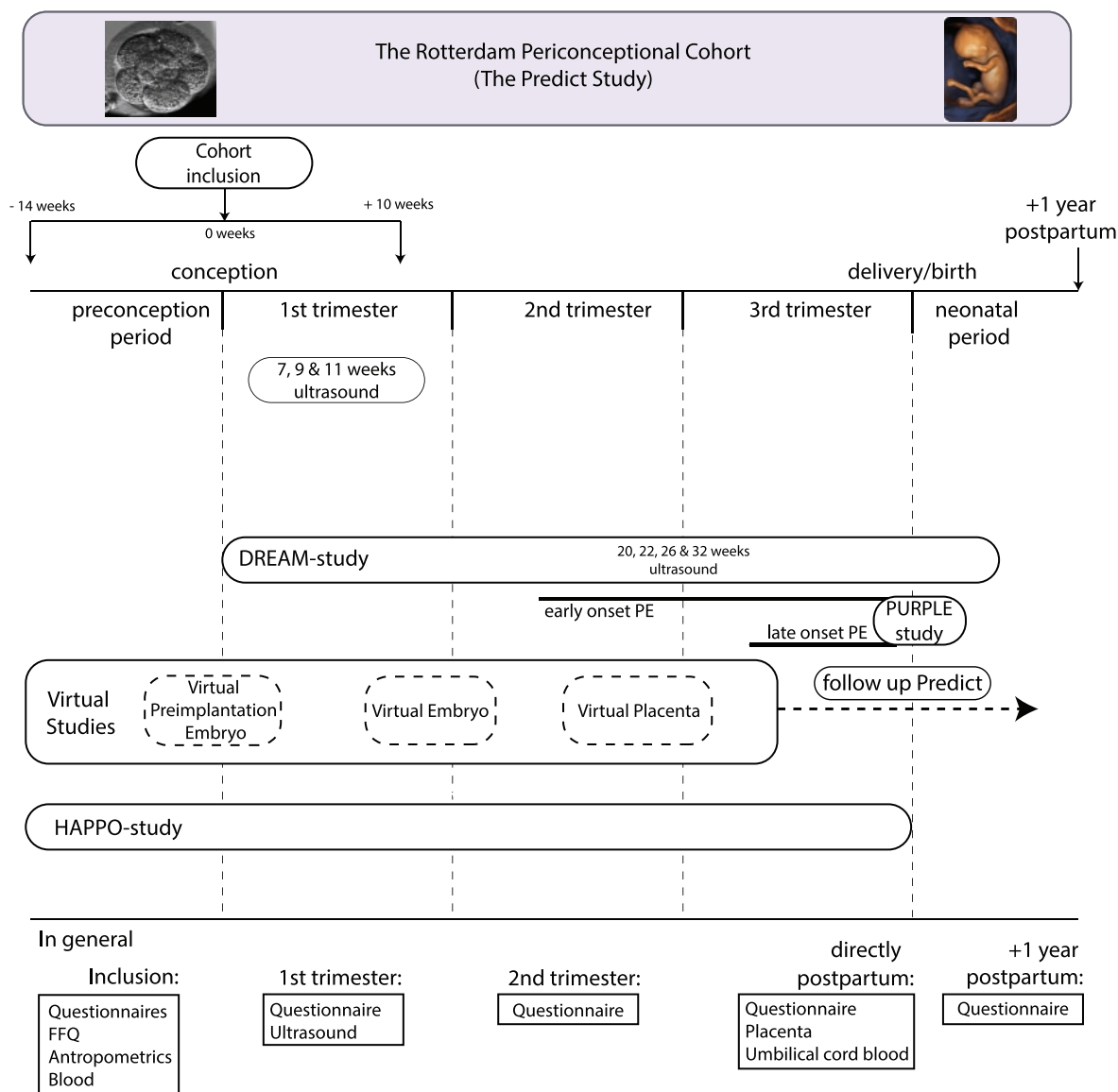
**Figure 1** Flowchart of included participants from November 2010 until August 2018 (without the pilot study participants). GA, gestational age

invited to participate (by means of an information brochure). Eligible couples undergo a standardized booking visit, in which height, weight, waist and hip circumference and systolic and diastolic blood pressure are determined. Before this booking visit, participants, including both women and their partners, are asked to fill out an extensive online questionnaire concerning both parental characteristics, medical (and obstetric) history, pre-pregnancy body mass index (BMI) and lifestyle behaviours. Moreover, a 196-item Food Frequency Questionnaire (FFQ) is completed for information about parental dietary patterns. Follow-up questionnaires are sent at 24 weeks of gestational age, around the expected delivery date and 1 year after actual delivery. Follow-up questionnaires collect information on health and environmental exposures and the course and outcome of pregnancy. One year after delivery, final information on general health of the offspring, including growth, congenital malformations and development is requested.

Preconceptionally and/or during the first trimester, both maternal and paternal venous blood samples are collected. At birth, umbilical cord blood is collected and, depending on the subcohort, the placenta is sent for pathological examination. [Table 1](#) shows the numbers and percentages of

available questionnaires and samples that have been collected up until August 2018 (excluding the pilot phase).

As mentioned previously, the Predict study serves as an infrastructure for embedding new subcohorts. The first subcohort study, the Dream study, focused on detailed markers of embryonic and fetal brain development in the first, second and third trimester of pregnancy and continuing into the neonatal period ( $N=227$ ).<sup>8–12</sup> The second subcohort study, the Purple study, aimed at the occurrence of early-onset and late-onset preeclampsia. Placenta and umbilical cord (postpartum) pathological examination, umbilical cord cell populations and the tissue-specific epigenome of the placenta and newborn were studied ( $N=448$ ).<sup>13–15</sup> The third subcohort study, the Virtual Placenta study, concentrated on placental growth and development starting as early as the first-trimester ( $N=241$ ).<sup>16</sup> Ongoing subcohorts are the Virtual Preimplantation Embryo study, focusing on (pre-implantation) embryonic growth and development from the preconception period onwards ( $N=360$ , number until 1 January 2019, still ongoing) and the HAPPO study, concentrating on maternal haemodynamic parameters before and during pregnancy ( $N=25$ , number until October 2019, still ongoing).<sup>17</sup>



**Figure 2** Study design of Predict Cohort; including all subcohorts. General investigations of all subcohorts during the preconception period, 1st, 2nd and 3rd trimester, postpartum period, including follow-up at 1 year after delivery are shown. PE, preeclampsia; FFQ, Food Frequency Questionnaire

In addition, intervention studies designed as survey and multicentre randomized controlled trials are conducted using the mHealth coaching platform Smarter Pregnancy to empower couples to adapt a healthier lifestyle from the periconceptional period onwards.<sup>18,19</sup> Also pregnant women with or without their male partner could participate in the study.<sup>20,21</sup>

**What is the reason for the new focus?**

Over the past years, data have been collected on 233 (pilot phase study) and 2717 (Predict study) couples and their pregnancies. Data have been used in multiple published articles in which several associations between exposures and both periconceptional and pregnancy outcomes have

been studied (Supplementary File 1, available as Supplementary data at IJE online).

This cohort profile update paper not only represents an update of these results, but also provides description and discussion of our 3D ultrasound and virtual reality measurement protocols. The results of the studies within the Predict cohort necessitated a re-evaluation and new strategies within the cohort. To optimize, further understand and unravel the potentials of maternal and paternal periconceptional health, an additional new study design of the original cohort is required. The new strategy and reason for the current update profile paper are aimed at studying the effects of an intervention on growth and development of the unborn child. Therefore, in 2021 the study will be extended with a nested randomized controlled trial, using

**Table 1** Data collection and general characteristics of the participants included between November 2010 and August 2018

Data collected	Women (N = 1744)	Men (N = 1557)
<b>First trimester</b>	<b>N (%)</b>	<b>N (%)</b>
General questionnaires	1535 (88.0)	1329 (85.4)
Food Frequency Questionnaires	1421 (81.5)	1186 (76.2)
Anthropometric measurements	1630 (93.5)	1331 (85.5)
Venous blood samples	1405 (80.6)	1272 (81.7)
(Serial) ultrasound scans	1285 (73.7)	NA
<b>24 weeks of gestation</b>		
Questionnaires	1149 (65.9)	NA
Structural ultrasound records	1278 (73.3)	
<b>36 weeks of gestation to delivery</b>		
Questionnaire	977 (56.0)	NA
Delivery reports	1101 (63.1)	NA
Umbilical cord blood	529 (30.3)	NA
Placenta	147 (8.4)	NA
<b>1 year after delivery</b>		
Questionnaires	756 (43.3)	NA
<b>General characteristics</b>	<b>Women</b>	<b>Men</b>
	Mean (SD) or N (%)	Mean (SD) or N (%)
Age, years	32.4 (4.5)	35.3 (6.1)
Geographical origin		
Dutch	1201 (80.0)	1091 (83.5)
Other Western	68 (4.5)	41 (3.1)
Non-Western	233 (15.5)	174 (13.3)
Education		
Low	126 (8.5)	181 (14.0)
Middle	529 (35.5)	474 (36.8)
High	835 (56.0)	634 (49.2)
BMI, kg/m <sup>2</sup>	25.9 (5.2)	26.4 (4.1)
Parity		NA
Nulliparous	678 (53.9)	
Multiparous	580 (46.1)	
Mode of conception		NA
Spontaneous	1037 (67.6)	
IVF/ICSI	496 (32.4)	
First trimester outcome		NA
Miscarriage	182 (10.4)	
Pregnancy outcome		NA
Birthweight, g	3270 (680)	
Gestational age at birth, days	273 (61)	
PIH/preeclampsia	117 (9.4)	
Preterm birth (<37weeks GA)	118 (9.5)	
Small for gestational age (<p10)	154 (12.3)	
Congenital anomaly	54 (4.3)	

SD, standard deviation; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; BMI, body mass index; PIH, pregnancy-induced hypertension; GA, gestational age.

Update until August 2018, since all participants delivered and we could include the pregnancy outcome data in this manuscript.

a periconceptional blended lifestyle care approach in a specific high-risk population (BMI >25 kg/m<sup>2</sup>). Apart from the previous mentioned intervention, the design will be as the general observational cohort. The nested randomized controlled trial is considered the best design to show

effectiveness, and has been developed to study the outcomes as defined for the cohort by integrating a blended lifestyle care approach into the current level of standardized health care.<sup>22</sup> The study will be conducted during the periconceptional period in women at high risk of

pregnancy complications and including their partners. The control group will receive standard care as also provided to the participants of the cohort according to local and national antenatal care protocols. The intervention group of high risk women will receive a combination of a personalized eHealth coaching programme for a period of 26 weeks [www.smarterpregnancy.co.uk] and a face-to-face counselling session with focus on lifestyle improvements. This approach allows for analyses of subgroups and associations between lifestyle changes and reproductive outcomes in the women, their male partners and their offspring.

To gain further insight into fetal and placental development, 3D ultrasound examinations at 24 and 32 weeks of gestation will additionally be performed among all participants.

### What will be the new areas of research?

Due to a 10-year anniversary evaluation, new areas in the dataset will be explored. In ongoing both uncomplicated and complicated pregnancies (i.e. miscarriage, intra-uterine fetal death, congenital anomalies, hypertensive disorders, large for gestational age, fetal growth restriction and preterm birth), serial parameters have been collected. Unique data are available for analysis since collection was already initiated during the periconceptional period. Until now, data have been published of subsets of these pregnancies focusing on the ongoing uncomplicated pregnancy group. In the existing dataset, as well as in new subcohorts, unique areas of research will be explored, by studying determinants of maternal and paternal periconceptional health in association with reproductive performance, pregnancy course and outcome, as well as underlying molecular biological mechanisms, including cardiovascular and metabolic pathways, inflammation and epigenetic profiles.

The Virtual Pre-implantation Embryo study investigates pre-implantation embryonic development using the EmbryoScope<sup>TM</sup>, a time-lapse incubator providing a controlled culture environment and capturing comprehensive information on morphokinetics of the pre-implantation embryo. In animal models, the timing of embryonic developmental events has been shown to reflect embryonic metabolism and genetic integrity.<sup>23</sup> The use of the EmbryoScope<sup>TM</sup> will enable us to further unravel the link between periconceptional parental exposures (such as obesity), *in vitro* environment, pre-implantation and post-implantation embryonic growth and development.

In collaboration with the Biomedical Imaging Group of the Erasmus MC, new innovative research will use deployment of artificial intelligence (AI) to focus on fully automated analyses of 3D ultrasound data, with the goal to

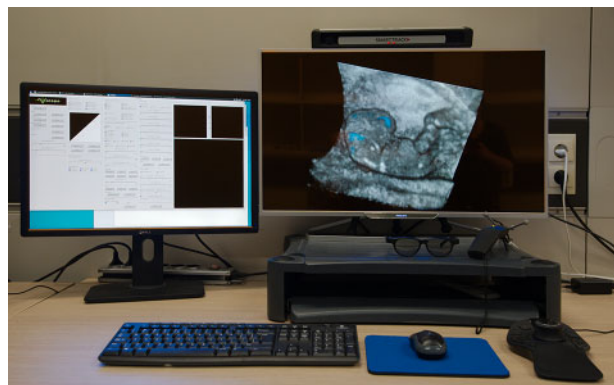


**Figure 3** I-Space virtual reality system. A fetus of 12 weeks' gestational age is displayed on the front wall. The researcher is wearing 3D glasses to observe real depth perception and interact with the dataset by using a joystick. Be aware that real depth perception cannot be shown on paper

develop a spatiotemporal 3D ultrasound embryonic and fetal brain atlas. The new 3D atlas will enable fully automated pattern recognition within the developing embryonic and fetal brain. The use of AI will enable more reliable, less time-consuming and more independent measurements of brain structures and allow for putting the development of the entire prenatal brain into perspective regarding different parental exposures as well as maternal, fetal and neonatal outcomes.

The maternal environment and its cardiovascular system adaptation to pregnancy are crucial in the development of the embryo, the fetus and the placenta. The general aim of the Virtual Placenta study is to study periconceptional and (patho)physiological maternal cardiovascular and uterine vascular placental (adaptation) mechanisms that contribute to the origin of placenta-related pregnancy outcomes. Within this study, first-trimester reference values for longitudinal uterine vascular placental parameters will be determined using 3D power Doppler ultrasound and virtual reality. To get more insight into vascular processes involved in *in utero* vascular placental development, placental parameters and embryonic growth trajectories, pregnancy outcome and periconceptional maternal vascular adaptation mechanisms will be studied. In addition to uterine vascular placental development studied using state-of-the-art 3D ultrasound and virtual reality techniques, maternal haemodynamic parameters before and during pregnancy are studied in the HAPPO study.

The HAPPO study investigates the haemodynamic adaptation of women to pregnancy by examination of the maternal macro-circulatory and micro-circulatory system and uterine vascular placental development in the preconception period, during pregnancy and 3 months after



**Figure 4** Three-dimensional virtual reality desktop system. An embryo of 9 weeks' gestational age is displayed on the 3D monitor; the brain cavities are coloured in light blue (and the brain cavity volume is measured automatically)

delivery. The main outcome measures are differences in maternal haemodynamic adaptation to pregnancy between women with and without placenta-related pregnancy complications. A more detailed description of the HAPPO study protocol has been published separately.<sup>17</sup>

Finally, we want to address Smarter pregnancy. This is an example of a platform that has been first studied in a research setting and is now being used in regular clinical care at our outpatient clinic [www.smarterpregnancy.co.uk].<sup>18,19</sup> The platform has been incorporated in the administrative database of the Erasmus MC since 2019; so every health care professional can see when a woman and her partner were included in this platform, together with the outcome of the visit and future goals. With permission of the woman and her partner, the information is also incorporated in national clinical and epidemiological databases.

### What are the new measurements?

Two-dimensional and 3D transvaginal and transabdominal ultrasound examinations are performed and subsequently analysed offline. Embryonic and fetal structures of interest include strictly defined and clinically important biometric measurements and also novel biometric and volumetric parameters. Examples of these novel parameters are first-trimester head volume and uterine vascular placental volume. Parameters still being developed are measurements of inner organ systems like the first-trimester kidney (length and volume) and lung (length and volume). [Table 2](#) provides an overview of structures for which reliability analysis has been performed. [Supplementary File 2](#), available as [Supplementary data](#) at *IJE* online, contains all measuring protocols of measurements as described in [Table 2](#).

Examinations are performed on a Voluson E8 or E10 (GE Healthcare, Austria) ultrasound machine. Measurements are carried out by ultrasound or on a personal computer using 4D view software (GE Healthcare, Austria). This software program allows offline 3D measurements after collection of the datasets and is only available for datasets generated on GE Healthcare ultrasound machines. The 3D measurements are performed on a 2D computer screen, not allowing real depth perception. In addition, complex and volumetric structures are measured using virtual reality techniques. The I-Space, one of the used systems, is a virtual reality room in which eight projectors project stereoscopic images on three walls and the floor of a small room, creating a so-called hologram of the embryo or fetus ([Figure 3](#)). V-Scope is the volume-rendering software package used for virtual reality measurements, allowing interaction with the dataset in an intuitive manner.<sup>24</sup> Within the I-Space, different structures are measured using a wireless joystick. Length measurements can be performed using a tracing tool, and (semi-)automated volume measurements use an algorithm based on ultrasound-related grey scale differences.<sup>25–48</sup> Finally, morphological examinations can be performed using all interaction modalities.<sup>49</sup> As the I-Space is a physically static, large and expensive system, a 3D virtual reality desktop system has been developed, using a 3D monitor and a tracking system ([Figure 4](#)). The software program used on the I-Space and virtual reality desktop system are the same (V-Scope), which means that the measurements are performed in the same exact way. However, the virtual reality desktop system is smaller in size, which entails measuring taking place on a smaller screen.

Importantly, the software has not changed during the previous years; only new modalities have been added. Baken *et al.* validated the desktop system for crown-rump length (CRL) and embryonic volume (EV) measurements in 30 patients, showing excellent reliability: intraclass correlation coefficient (ICC) CRL 1.000 (95% confidence interval (CI): 0.999–1.000) and ICC EV 0.999 (95% CI: 0.998–1.000).<sup>50,51</sup> Since the 3D virtual reality desktop system uses exactly the same software program, we did not publish reliability data regarding other structures if these were measured on the desktop system instead of the I-Space. However, each researcher who is involved in the ultrasound measurements is obliged to pass the learning curve set in order to test their reproducibility of the measurements. Assessment of the intra-observer reliability involves measuring the structure of interest three times. Inter-observer reliability is assessed by comparing with multiple measurements previously performed in the I-Space or on the desktop system by other researchers. Only when an excellent reliability (intraclass correlation

**Table 2** Reliability of different (newly) introduced measures performed using 3D ultrasound and virtual reality techniques

Article	Year	Measures	Technique(s) used	Intraclass correlation coefficient (95% CI)	Mean difference	95% CI for mean difference	Limits of agreement	Year of collection	Gestational weeks	Number of datasets
<i>Verwoerd et al.</i>	2008	Crown-rump length (mm)	4D View, I-Space <sup>a</sup>	1,000 (0.982–0.997)	-0.07	-0.32 to 0.18	-1.37 to 1.22	2008	6 to 14	28
		Biparietal diameter (mm)	4D View, I-Space <sup>a</sup>	0.987 (0.971–0.994)	-0.47	-0.99 to 0.05	-3.06 to 2.11	2008	6 to 14	26
		Head circumference (mm)	4D View, I-Space <sup>a</sup>	0.994 (0.987–0.997)	-1.07	-2.17 to 0.04	-6.58 to 4.45	2008	6 to 14	26
		Abdominal circumference (mm)	4D View, I-Space <sup>a</sup>	0.998 (0.997–0.999)	-0.49	-0.95 to -0.04	-2.76 to 1.77	2008	6 to 14	26
<i>Rousian et al.</i>	2009	Yolk sac diameter (mm)	4D View, I-Space <sup>a</sup>	0.993 (0.982–0.997)	-0.04	-0.09 to 0.02	-0.29 to 0.22	2008	6 to 14	20
		Yolk sac volume (cm <sup>3</sup> )	VOCAL, inversion mode, SonoAVC, I-Space <sup>e</sup>	0.992 (0.981–0.996)	-0.00	-0.00 to 0.00	-0.005 to 0.004	2008	6 to 11	24
<i>Rousian et al.</i>	2010	Balloons (cm <sup>3</sup> )	VOCAL, inversion mode, SonoAVC, I-Space <sup>e</sup>	0.997 (0.982–0.999)	-0.01	-0.03 to 0.00	NA	2008	NA	7
		Embryonic volume	I-Space	0.999 (0.997–0.999)	NA	NA	NA	2008	6 to 12	20
		Trophoblast volume (cm <sup>3</sup> )	VOCAL	0.976 (0.945–0.989)	1.57	-0.81 to 3.94	-9.69 to 12.82	2009	6 to 11	24
		Total cerebellar diameter (mm)	4D View, I-Space <sup>a</sup>	0.996 (0.986–0.999)	-0.12	-0.18 to -0.06	-0.45 to 0.22	2009	7 to 12	35
<i>Baken et al.</i>	2014	Left hemispheric diameter (mm)	4D View, I-Space <sup>a</sup>	0.992 (0.984–0.996)	-0.02	-0.06 to 0.02	-0.26 to 0.23	2009	7 to 12	35
		Left hemispheric thickness (mm)	4D View, I-Space <sup>a</sup>	0.985 (0.968–0.992)	-0.04	-0.09 to 0.00	-0.31 to 0.22	2009	7 to 12	33
		Right hemispheric diameter (mm)	4D View, I-Space <sup>a</sup>	0.985 (0.970–0.992)	-0.03	-0.09 to 0.03	-0.37 to 0.31	2009	7 to 12	35
		Right hemispheric thickness (mm)	4D View, I-Space <sup>a</sup>	0.986 (0.971–0.993)	-0.05	-0.09 to 0.00	-0.29 to 0.20	2009	7 to 12	34
<i>Gijtenbeek et al.</i>	2014	Wrist width (mm)	I-Space	0.984 (0.960–0.994)	-1.47	-4.08 to 11.40	-12.40 to 9.46	2009	9 to 12	20
		Hand width (mm)	I-Space	0.992 (0.981–0.997)	-1.14	-3.02 to 0.96	-9.95 to 7.67	2009	9 to 12	20
		Hand length (mm)	I-Space	0.997 (0.994–0.999)	-0.25	-1.94 to 1.43	-7.31 to 6.81	2009	9 to 12	20
		Hand index	I-Space	0.984 (0.956–0.993)	-0.86	-2.88 to 1.16	-9.34 to 7.62	2009	9 to 12	20
<i>Rousian et al.</i>	2014	Left thickness diencephalon (mm)	4D View	0.999 (0.998–0.999)	-0.01	-0.03 to 0.01	-0.12 to 0.11	2009	6 to 12	30
		Right thickness diencephalon (mm)	4D View	0.997 (0.994–0.999)	-0.03	-0.06 to 0.00	-0.19 to 0.14	2009	6 to 12	30
		Total diencephalon diameter (mm)	4D View	0.998 (0.996–0.999)	0.04	-0.00 to 0.08	-0.19 to 0.27	2009	6 to 12	30
		Left thickness mesencephalon (mm)	4D View	0.992 (0.982–0.996)	-0.03	-0.06 to 0.00	-0.20 to 0.14	2009	6 to 12	30
<i>Verwoerd et al.</i>	2008	Right thickness mesencephalon (mm)	4D View	0.988 (0.974–0.994)	0.00	-0.04 to 0.04	-0.21 to 0.21	2009	6 to 12	30

(Continued)



Table 2 Continued

Article	Year	Measures	Technique(s) used	Intraclass correlation coefficient (95% CI)	Mean difference	95% CI for mean difference	Limits of agreement	Year of collection	Gestational weeks	Number of datasets
		Right thickness mesencephalon (mm)								
		Total mesencephalon diameter (mm)	4D View	0.994 (0.987–0.997)	0.05	-0.01 to 0.11	-0.27 to 0.37	2009	6 to 12	30
		Left telencephalon thickness (mm)	4D View	0.980 (0.950–0.991)	-0.05	-0.09 to -0.01	-0.26 to 0.16	2009	6 to 12	30
		Right telencephalon thickness (mm)	4D View	0.982 (0.963–0.992)	0.03	-0.01 to 0.07	-0.20 to 0.26	2009	6 to 12	30
Reus <i>et al.</i>	2014	Trophoblast volume (cm <sup>3</sup> )	I-Space	0.928 (0.829–0.971)	3.88	-1.41 to 9.17	-18.74 to 26.49	Nov 2009–Dec 2010	12	20
		Uteroplacental bed vascular volume (cm <sup>3</sup> )	I-Space	0.994 (0.985–0.998)	-0.64	-1.25 to -0.03	-3.23 to 1.95	Nov 2009–Dec 2010	12	20
		Fetal vascular volume (cm <sup>3</sup> )	I-Space	0.964 (0.913–0.986)	0.18	-0.04 to 0.40	-0.74 to 1.10	Nov 2009–Dec 2010	12	20
Baken <i>et al.</i>	2015	Crown-rump length (mm)	I-Space VS 3 D VR Desktop	1.000 (0.999–1.000)	-0.34	-0.77 to 0.08	-2.58 to 1.89	Jan 2009–Dec 2009	6 to 12	30
		Embryonic volume (cm <sup>3</sup> )	I-Space VS 3 D VR Desktop	0.999 (0.998–1.000)	-0.92	-2.07 to 0.23	-6.97 to 5.13	Jan 2009–Dec 2009	6 to 12	30
<b>Cohort profile update measurements</b>										
Koning <i>et al.</i>	2016	Head volume (cm <sup>3</sup> )	I-Space	0.99 (NA)	0.01	-0.03 to 0.05	-7.56 to 6.74	2009–10	9 to 12	34
Koning <i>et al.</i>	2017	Sylvian fissure depth (mm)	4D view	0.846 (NA)	0.51	0.13 to 0.89	-2.18 to 3.19	Nov 2013–March 2015	22 to 32	30
		Insula depth (mm)	4D view	0.879 (NA)	0.31	-0.12 to 0.74	-2.73 to 3.35	Nov 2013–March 2015	22 to 32	30
		Parieto-occipital fissure depth (mm)	4D view	0.841 (NA)	-0.48	-1.05 to 0.09	-4.44 to 3.91	Nov 2013–March 2015	22 to 32	30
Koning <i>et al.</i>	2017	Corpus callosum length (mm)	4D view	0.970 (NA)	-1.11	-1.70 to -0.51	-4.55 to 2.33	Nov 2013–July 2015	22 to 42	30
		Corpus callosum fastigium length (mm)	4D view	0.970 (NA)	-0.13	-0.74 to 0.49	-3.59 to 3.34	Nov 2013–July 2015	22 to 42	30
Roelants <i>et al.</i>	2017	Fractional thigh volume (cm <sup>3</sup> )	4D View	Excellent	NA	NA	NA	Nov 2013–July 2015	22 to 32	30

(Continued)

Table 2 Continued

Article	Year	Measures	Technique(s) used	Intraclass correlation coefficient (95% CI)	Mean difference	95% CI for mean difference	Limits of agreement	Year of collection	Gestational weeks	Number of datasets
Bogers <i>et al.</i>	2018	Genital tubercle angle	I-Space	0.95 (0.90–0.98)	NA	NA	NA	2009	9 to 12	30
		Qualitative sex prediction <sup>b</sup>	I-Space	NA	NA	NA	NA	2009	9 to 12	30
Bogers <i>et al.</i>	2018	Umbilical cord insertion width (mm)	I-Space	0.917 (NA)	0.01	-0.08 to 0.10	-0.48 to 0.49	2009	6 to 12	31
		Maximum diameter umbilical cord (mm)	I-Space	0.962 (NA)	0.04	-0.04 to 0.12	-0.40 to 0.48	2009	6 to 12	31
		Volume midgut herniation (cm <sup>3</sup> )	I-Space	0.997 (NA)	0.29	-0.46 to 1.03	-3.75 to 4.32	2009	6 to 12	31
	2018	Foot position—left frontal	I-Space	0.935 (NA)	0.14	-10.56 to 10.85	NA	2009	8 to 13	30
		Foot position—right frontal	I-Space	0.941 (NA)	0.33	-9.28 to 9.95	NA	2009	8 to 13	30
		Foot position—left lateral	I-Space	0.872 (NA)	-0.39	-6.38 to 5.60	NA	2009	8 to 13	30
		Foot position—right lateral	I-Space	0.909 (NA)	0.57	-6.17 to 7.32	NA	2009	8 to 13	30
Reijnders <i>et al.</i>	2018	Uterine vascular volume (cm <sup>3</sup> ) preconceptional	I-Space, <sup>a</sup> 3 D VR Desktop	0.93 (NA)	-0.27	-0.55 to 0.01	NA	NA	7 to 11	35
		Uteroplacental vascular volume (cm <sup>3</sup> ) at 11 weeks GA	I-Space, <sup>a</sup> 3 D VR Desktop	0.69 (NA)	2.56	0.90 to 4.21	NA	NA	7 to 11	35
		Embryonic vascular volume (cm <sup>3</sup> ) at 11 weeks GA	I-Space, <sup>a</sup> 3 D VR Desktop	0.99 (NA)	0.00	-0.05 to 0.06	NA	NA	7 to 11	35

Intraclass correlation coefficient, mean difference and limits of agreement for interobserver differences (between the measurements performed by two independent examiners).

GA, gestational age; NA, not available.

<sup>a</sup>The results are shown for the I-Space measurements.

<sup>b</sup>Cohen's kappa = 0.24 (95% CI: 0.13–0.34).

<sup>c</sup>I-Space measurements are compared with the golden standard, namely VOCAL.

coefficient  $>0.90$ ) is met, the researcher can start measuring the structure of interest in the research set.

Complex structures, like brain cavity volume, Sylvian fissure depth, genital tubercle angle and different dimensions of the cerebellum can also be measured with high ICCs.<sup>10,33,43,44</sup> Table 2 shows the mean difference (including 95% CI), limits of agreement between observers, gestational age of measurement performance and number of datasets used for various morphological, biometric and volumetric structures. For each study parameter, different pregnancies were used to perform the reliability analysis. Pregnancies were randomly chosen based on gestational age and only one dataset per pregnancy could be included in the measuring set, to exclude selection bias. During the 10-year period a considerable amount of experience was gained, and we have learned to sophisticate our ultrasound examinations and study protocols in such a way that, in case of specific, small structures, additional 3D sweeps are necessary to measure the specific structure of interest. Therefore we included new pregnancy datasets when measuring these structures. After proving that these structures could be reliably measured, they are being measured in a larger subset.

## What has been found? Key findings and publications

To date, numerous original papers have been published including reliability analyses of newly introduced measurements (Table 2).

### Determinants of maternal and paternal periconceptional health

We studied associations between several exposures, such as maternal age, diet, smoking and BMI, and embryonic (CRL, EV, Carnegie stages), brain (head volume, cerebellum, cortical folding, corpus callosum, corpus callosum fastigium) and placental development (uterine vascular placental volume).<sup>52–64</sup> Supplementary File 1 shows the results of various studies with regard to these domains of intrauterine growth and development.

Koning *et al.* showed that congenital heart defects are associated with cortical folding (growth trajectories of left insula depth and right parieto-occipital fissure depths were lower).<sup>10</sup> Fractional thigh volume, measured in the fetal period using 3D ultrasound, showed to be a promising marker for prediction of neonatal adiposity.<sup>65</sup> Finally, in a subset of women with preeclampsia, a nested case-control study was performed consisting of 15 patients with early-onset and 15 with late-onset preeclampsia, respectively.<sup>14,15</sup> Placental and newborn vascular health was

studied, showing associations between preeclampsia and (a smaller) umbilical vein area and wall thickness. These parameters serve as a proxy of disturbed cardiovascular development within the newborn.

### Reproductive performance and pregnancy course and outcome

The link between parental periconceptional exposures and fertility parameters has been studied. A positive association between strong adherence to a healthy dietary pattern and semen parameters in men with poor semen quality has been shown.<sup>66</sup> With regards to female fertility, strong periconceptional adherence to a healthy diet showed an inverse association with the hyperandrogenic polycystic ovarian syndrome (PCOS) phenotype. In women with PCOS, a strong periconceptional adherence to a healthy dietary pattern showed a 3-fold higher chance of an ongoing pregnancy.<sup>67</sup> We also focused on endocrine and cardiometabolic cord blood characteristics of PCOS offspring, and compared these with characteristics of healthy controls.<sup>68</sup> Androstenedione concentrations were increased in cord blood of both male and female PCOS offspring, supporting the hypothesis that maternal hyperandrogenism during pregnancy may predispose to fetal hyperandrogenism.

### Underlying epigenetic profiles

Herzog *et al.* studied epigenetic programming of placental and fetal tissues in women with early-onset and late-onset preeclampsia, and identified loci known to be associated with cardiovascular system pathways.<sup>14,15</sup> In these women, umbilical cord blood cell populations collected postpartum showed derangements of fetal haematopoiesis, in particular of neutrophil and nucleated red blood cell counts. The heterogeneity in umbilical cord blood cell populations should be considered a confounder in epigenetic association studies.<sup>13</sup> A methylation study of umbilical cord blood leukocytes by van den Berg *et al.* in early-onset preeclampsia demonstrated differences in DNA methylation of circadian clock and clock-controlled genes.<sup>69,70</sup>

### What are the main strengths and weaknesses?

The study design, being a unique periconceptional prospective cohort study, fully embedded in standard (tertiary) patient care, is the main strength of the study. Besides periconceptional questionnaires and biomarkers, study parameters include longitudinal 2D and 3D ultrasound examinations already from 6+0 weeks of gestation

onwards. Measurements are performed using high-frequency ultrasound probes and virtual reality, allowing visualization and innovative measurement of structures for the first time. Besides virtual reality imaging techniques, biochemical, dietary, molecular biological and (epi)genetic data are available for integrated analysis, contributing to our further understanding of the epigenome as a predictor of reproductive and pregnancy outcomes.

Since we are interested in understanding the causation of complicated reproduction and pregnancy outcome, the cohort is embedded in tertiary care, which ensures a high internal validity but a limited external generalizability. All findings emphasize the importance of implementing periconceptional care in general medicine in order to prevent and ameliorate adverse pregnancy and future health outcomes in the offspring. Although the Predict study is a prospective cohort study, a validated intervention is needed to elucidate and enrich the findings.

### Can I get hold of the data? Where can I find out more?

Colleagues interested in collaboration can contact us by sending a brief research proposal ([Supplementary File 3](#), available as [Supplementary data](#) at *IJE* online). Approval depends on quality and feasibility, as assessed by the Principal Investigators of the Predict study. A formal contract is needed for collaboration, including mutual obligations. For more information contact the Principal Investigators at [[predictstudie@erasmusmc.nl](mailto:predictstudie@erasmusmc.nl)]; and for possibilities regarding harmonization of data go to [[www.maelstrom-research.org](http://www.maelstrom-research.org)].

### Supplementary data

[Supplementary data](#) are available at *IJE* online.

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### Ethics approval

The study was approved by the Central Committee on Research in The Hague and the local Medical Ethics Committee of the Erasmus Medical Center (MC) in Rotterdam, The Netherlands (MEC-2004-227).

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### Conflict of interest

None declared.

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