



## Cohort Profile

# Cohort Profile: The Rotterdam Periconceptual Cohort (Predict Study)

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## Why was the cohort set up?

Interactions between parental conditions, environmental exposures and genetic variations may affect growth, development, plasticity and functioning of parental gametes and reproductive (extra-) embryonic and fetal organs. Evidence is compelling that environmental exposures during pregnancy also contribute to obesity and non-communicable diseases (NCD) during the life course.<sup>1</sup> Newborns with fetal growth restriction are at risk for developing cardiovascular, metabolic, endocrine and psychiatric diseases in adulthood. Accumulating data also indicate increased risks of vascular and metabolic diseases in women who experienced pregnancy complications, such as pregnancy-induced hypertension, preeclampsia and gestational diabetes.<sup>1,2</sup> Accordingly, pregnancy can be considered as a stress test for metabolic, endocrine, vascular and (neuro)psychological functions.<sup>3–6</sup>

Most reproductive failures, pregnancy complications and adverse pregnancy outcomes originate in the periconceptual period, a time window of 14 weeks before and up to 10 weeks after conception.<sup>7</sup> So far most birth cohorts started with enrolment and data collection after the first trimester of pregnancy, thereby ignoring the periconceptual window. In order to predict these periconceptual outcomes in the future, we initiated the Rotterdam

Periconceptual Cohort (Predict Study) designed as a hospital-based prospective open birth cohort study.

The focus of the Predict Study is on three research areas:

- i. The determinants of periconceptual health of the couple (general health conditions, nutrition, lifestyle and environment);
- ii. the reproductive performance and pregnancy course and outcome (gamete and embryo quality, (extra-) embryonic and fetal growth trajectories and development, infants health at birth and the first year of life);
- iii. the underlying epigenetic profiles to explain associations between periconceptual parental health determinants, reproductive performance and pregnancy course and outcome.

This ongoing cohort study started in November 2010 and is conducted in collaboration with the departments of Bioinformatics, Biostatistics, Internal Medicine and Pharmacy and the Laboratory of Clinical Chemistry of the Erasmus MC, University Medical Center in Rotterdam, The Netherlands.

The study was approved by the Central Committee on Research in The Hague and the local Medical Ethical Committee of the Erasmus MC in Rotterdam,

The Netherlands (MEC-2004-227) and funded by the Department of Obstetrics and Gynaecology at the Erasmus MC.

### Who is in the cohort?

Approximately 6000 couples per year in the Rotterdam region are contemplating pregnancy. Around 2500 of them annually visit the Erasmus MC outpatient clinic, a tertiary hospital for high risk pregnancies. The Predict Study has a yearly capacity for first trimester serial ultrasound measurements of 250 pregnancies. In the pilot phase we included 233 pregnancies preconceptionally up to 8 weeks of gestation. After extending the inclusion window up to the 13th week of gestation, we periconceptionally enrolled 1228 couples in the open and ongoing cohort between November 2010 and April 2015, with subsequent follow-up of 792 pregnancies.

All women less than 13 weeks of gestation, scheduled for a first antenatal visit at the outpatient clinic of the Department of Obstetrics and Gynaecology at Erasmus MC, are invited with their partner to participate, by means of a brochure.<sup>8</sup> If a couple are willing to take part, eligibility is checked and an appointment for a standardized intake followed by a 3D ultrasound examination is scheduled. Eligibility criteria are that the woman and her partner have to be at least 18 years of age and familiar with the Dutch language in speaking and reading. In the preparation of the intake appointment, the couple receive detailed information about the Predict Study, informed consent forms and questionnaires to be filled out at home. The signed informed consent forms and completed questionnaires are checked at the intake appointment. None of the participants is offered incentives other than pictures of the serial ultrasound examinations.

Here we evaluate 4 years and 5 months of enrolment and follow-up (see [supplementary material](#), available as [Supplementary data](#) at *IJE* online). The response rate among the preconceptionally invited group was 61.8%. The response rate in the first trimester invited group is unknown, because the denominator of eligible patients cannot be determined. We included 1128 couples: 453 (40.1%) participated from preconception onwards, 377 (33.4%) before 8 weeks of gestation and 298 (26.4%) entered the study between 8 and 13 weeks of gestation. So far, in the preconception group 117 pregnancies have occurred. In total 494 couples (117 of the preconceptional group and 377 of the early pregnancy group) had a standardized intake visit at the outpatient clinic before 8 weeks of gestation and 298 couples were seen before 13 weeks of gestation. In 470 (95.1%) pregnancies out of the 494 preconception and early pregnancy group, serial 3D

ultrasound examinations were performed, of which 93 were in the pregnancies of the preconception group. Our target is follow-up of at least 1000 pregnancies with serial 3D ultrasound examinations until 1 year after birth.

### How often have they been followed up?

At the time of enrolment, couples complete a self-administered general and food frequency questionnaire at home, [Figure 1 \(Table 1\)](#). During the standardized intake visit at the hospital, experienced researchers and research nurses check these questionnaires in a standardized manner for completeness and consistency, perform the measurements and obtain venous blood samples from the couple.

The woman receives a second and third short questionnaire by mail at 24 and 36 weeks of gestation, to obtain information on health, exposures and the course and outcome of pregnancy ([Table 1](#)).

From 2011 we started sending all participating women a delivery package at 32 weeks of gestation, for the collection and preservation of umbilical venous cord blood immediately after birth. A visual instruction card explains the procedure to the attending midwife or gynaecologist for obtaining blood samples.

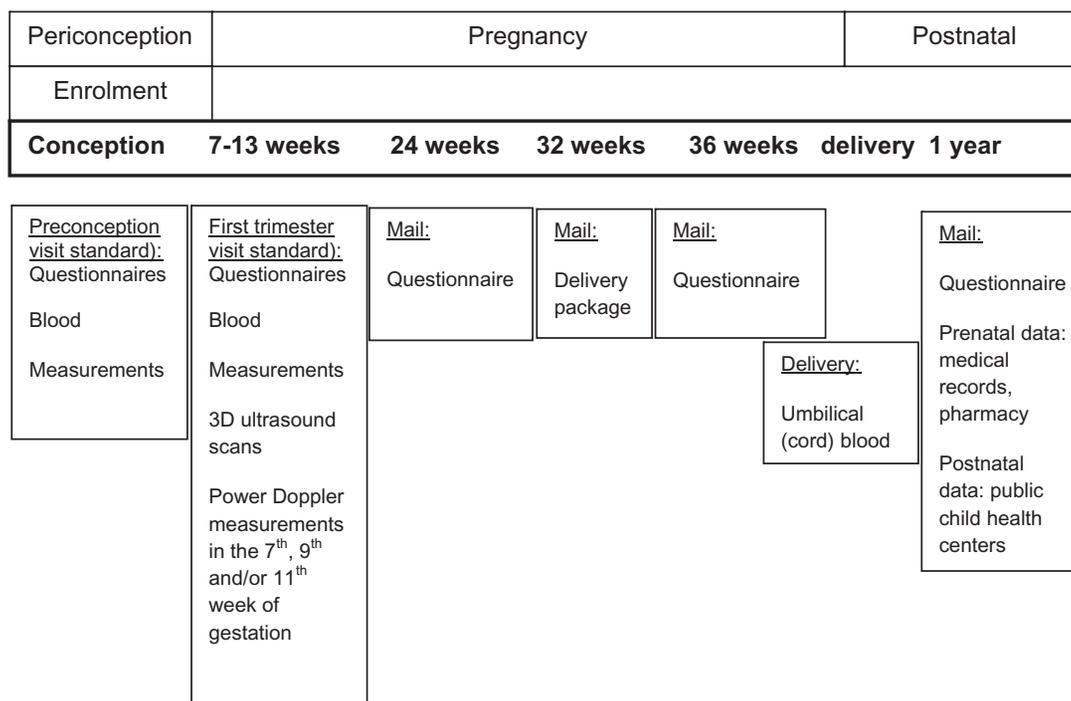
One year after delivery, mothers receive a final questionnaire by mail to collect information on general health, growth, congenital malformations and development of the infant.

To secure an accurate follow-up after inclusion, we developed a sophisticated digital monitoring system and study database consisting of personal details of each participant, questionnaire data and logistical data, e.g. date of the standardized intake visit, information on the sampling of materials from the participants and dates of sending and receiving questionnaires and reminders. Access to the database is granted based on the 'user role' of the database (data entry, statistician, study coordinator). All inserts and changes of data are logged in an automated audit system.

We plan to apply for funding to extend the follow-up period of parents and offspring.

### What has been measured?

[Table 2](#) shows the numbers and percentages of questionnaires and blood samples being collected. The intake questionnaire contains information from the couple on constitution, general health and medical and (non-) modifiable environmental exposures. We assess pregnancy dating in much detail. Data as to the first day of the last menstrual period (LMP) and regularity and duration of the menstrual cycle are obtained in the personal interview at the intake visit. We calculate gestational age from the LMP in



**Figure 1.** Periconceptional enrolment, collection of data and materials and follow-up.

spontaneously conceived pregnancies, from the date of oocyte pick-up plus 14 days in pregnancies conceived through *in vitro* fertilization with or without intra-cytoplasmic sperm injection (IVF/ICSI) procedures, from the LMP or insemination date plus 14 days in pregnancies conceived through intra-uterine insemination (IUI) and from the day of embryo transfer plus 17 or 18 days in pregnancies originating from transfer of cryopreserved embryos, depending on the number of days between oocyte pick-up and cryopreservation of the embryo. When the menstrual cycle is regular but more than 3 days different from 28 ( $28 \pm >3$  days), we adjust the gestational age for the duration of the menstrual cycle. If the LMP is missing or the difference between gestational age determined by crown-rump length (CRL) and LMP is more than 7 days, gestational age is based on the first CRL measurement in the I space after 8 weeks' gestational age.

We are using a standardized validated semi-quantitative food frequency questionnaire (FFQ) to collect detailed information on dietary intake.<sup>9-12</sup> The FFQ covers the food intake of the couple during the previous month. The amounts of foods consumed are estimated in commonly used units by using household measures. The FFQs are checked in a standardized manner for completeness and consistency during the intake appointment.

The questionnaires sent at 24 weeks and 36 weeks are used to obtain information on the use of folic acid, other vitamin supplements, drugs, lifestyle factors, participation in prenatal screening programmes in the first trimester,

fetal anomaly scanning at 20 weeks and the occurrence of complications during pregnancy and pregnancy outcome. Information on pregnancy complications, mode of delivery, gestational age, birthweight and the conditions of the mother and infant are retrieved from the medical records.

The questionnaire sent 1 year after delivery provides data on general health, growth, congenital malformations and medical history of the infant. The medical and medication data are verified using the medical records and records of the pharmacies. Prenatal data of fetal anomaly scanning at 20 weeks of gestation and the postnatal data from the public child health centres at which each child in The Netherlands is regularly checked on growth and development are also obtained.

The measurements of the couple comprise height and weight (without coat and shoes) to calculate the body mass index. Height and waist- and hip-circumference are measured. Systolic and diastolic blood pressure (left arm and in sitting position) are measured (Speidel & Keller, Welch Allyn).

Venous blood samples are obtained before conception and/or before 13 weeks of gestation for: (i) serum concentrations of red blood cell folate, serum vitamin B<sub>12</sub> and plasma total homocysteine; and (ii) biobanking for (epi)genetic and epidemiological research. In total 27 ml venous blood per individual is collected in three ethylenediamine tetraacetic acid (EDTA) plasma tubes and two serum separator tubes. Laboratory results are communicated to the couple.

**Table 1.** Details of data and material collection from the periconceptional period up to 1 year after delivery

	Preconception and first trimester	24 weeks of gestation	36 weeks of gestation to delivery	1 year after delivery
Conditions	Age Marital status Ethnicity Education			
Health	Diseases Medicine use Reproduction Andrological Psychological stress	Diseases Medicine use Reproduction Psychological stress	Diseases Medicine use Reproduction Psychological stress	Growth Development Malformations Medical information
Modifiable	Lifestyle (smoking, drugs, alcohol) Vitamins Infections	Lifestyle (smoking, drugs, alcohol) Vitamins Infections	Lifestyle (smoking, drugs, alcohol) Vitamins Infections	
Non or partially modifiable Family	Food Frequency Questionnaire Environmental and occupational exposures	Environmental and occupational exposures	Environmental-and occupational exposures	
Venous blood	Diseases Malformations White blood cell count and differentiation biomarkers (folate, B <sub>12</sub> , homocysteine) biobanking		Umbilical cord blood: blood and white blood cell counts and differentiation, haematopoietic stem cell biobanking	
Measurements	Systolic-diastolic blood pressure Height and weight Waist-hip circumference		Birthweight Placental weight Umbilical cord vessels	
Ultrasound	Serial 2D/3D ultrasound scans at 7, 9 and 11 weeks of gestation	Structural ultrasound scans at 20 weeks of gestation		
Verification		Medical-pharmacy records	Medical-pharmacy records	Medical-pharmacy records

**Table 2.** Collected data of the ongoing open cohort between November 2010 and April 2015 from all couples with a standardized visit in the first trimester of pregnancy ( $N=792$ )

Data collected	Women	Men
<b>First trimester</b>		
General questionnaires	645 (81.4%)	509 (64.3%)
Food Frequency Questionnaires	653 (82.5%)	648 (81.9%)
Venous blood samples	732 (92.5%)	625 (78.9%)
Serial ultrasound scans	563 (71.1%)	na
<b>24 weeks of gestation</b>		
Questionnaires	589 (74.4%) <sup>a</sup>	na
<b>36 weeks of gestation to delivery</b>		
Questionnaire	473 (59.7%) <sup>b</sup>	na
Umbilical cord blood	339 (42.8%) <sup>b</sup>	na
<b>1 year after delivery</b>		
Questionnaires	455 (57.4%) <sup>c</sup>	na

na, not available.

<sup>a</sup>Not all included women are  $\geq 24$  weeks of gestation

<sup>b</sup>Not all included women are being delivered.

<sup>c</sup>Not all included women are 1 year after delivery

At delivery, umbilical cord blood samples are obtained according to a protocol (see supplement, available as [Supplementary data](#) at *IJE* online). Umbilical venous cord blood is collected in one EDTA tube (10 ml) and one separator tube (10 ml). Immediately after delivery, white blood cell count and differentiation are determined in the umbilical cord blood sample within 48 h after collection.

All blood samples are stored  $-80^{\circ}\text{C}$  with barcodes in the biobank of the Predict Study situated at the laboratory of Clinical Chemistry of the Erasmus MC in Rotterdam for future research.

At the start of the study, pregnant women participating in the first trimester were eligible to receive weekly three-dimensional (3D) ultrasound scans. It was revealed from the pilot study that we could reduce the number of scans for accurate modelling of embryonic growth curves.<sup>13–15</sup> Therefore, we reduced the number of scans to three, performed in the 7th, 9th and 11th week of gestation at the end of 2012. These ultrasound scans are carried out by experienced researchers using a 6–12 MHz transvaginal probe of the GE Voluson E8 Expert system (GE, Zipf, Austria). The evaluation of the ultrasound scans focuses on the embryo, including details such as CRL, embryonic volume (EV) and curvature, brain structures and external Carnegie Stage characteristics<sup>16–18</sup> and extra-embryonic structures such as the gestational sac, umbilical cord,<sup>19,20</sup> placenta and yolk sac. In the first year of the study, the uterine artery and placental flow were documented by standardized 3D Power Doppler sweeps at the 11 weeks scan for offline evaluation. Power Doppler flow imaging is also used to determine placenta vascularization by measuring the volume of the blood vessels using our Barco I-Space

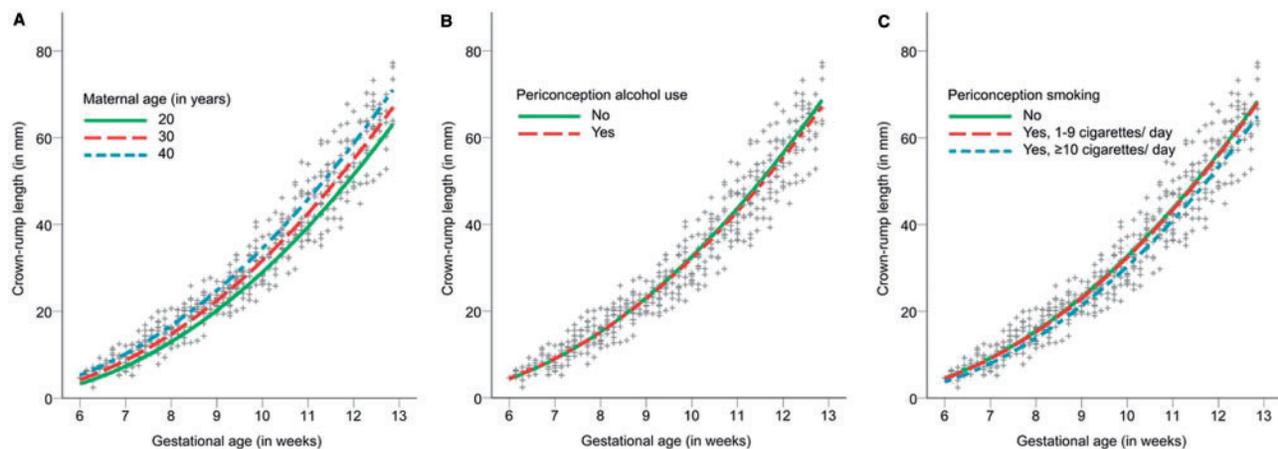
virtual reality (VR) system.<sup>21</sup> All 3D ultrasound scans are stored as Cartesian volumes using specialized 3D software (4D View, version 9.1, GE Medical Systems) and visualized using the I-Space, a four-walled CAVE<sup>TM</sup>-like<sup>22</sup> (Automatic Virtual Environment) virtual reality system built by Barco N.V. (Kuurne, Belgium). The V-Scope volume-rendering application creates an interactive 3D hologram of the ultrasound image that can be manipulated by means of a virtual pointer, projected from a wireless joystick.<sup>23</sup> The V-scope software used in the I-Space allows a more precise determination of the embryonic CRL innovative volume measurements and visualization of brain structures.<sup>24</sup> The V-Scope software is reliable and reproducible.<sup>16–18</sup>

Detailed measurements in the I-Space and using ultrasound with 4D view are performed by researchers according to a standard protocol. Following training, reproducibility of the measurements is determined. A random subset of five patients with six serial datasets (30 datasets) is provided and triplo measurements of the specific (extra-)embryonic structures are carried out. An inter- and intrameasurement variation of less than 5% and intraobserver and interobserver agreement of  $>0.90$  and  $P < 0.05$  must be achieved before measurements of the specific structure are performed. This is done by calculating intraclass and interclass correlation coefficients and Bland Altman plots with a mean percentage difference and 95% limits of agreement (mean percentage difference  $<5\%$  and limits  $<10\%$ ).

## What has been found? Key findings and publications

From the pilot study of 259 pregnancies including 73 IVF/ICSI pregnancies, it was revealed that early and late first-trimester CRL is associated with mid-pregnancy fetal growth, and CRL from 10 to 13 weeks of gestation (late first-trimester growth) is associated with birthweight.<sup>13</sup> Maternal age is shown to be associated with an increased CRL, and alcohol consumption and smoking with a decreased CRL, a measurement used to estimate first-trimester growth as illustrated in [Figure 2](#).<sup>14</sup> A very high ( $>1813$  nmol/l) maternal periconceptional folate status is associated with a smaller embryonic size.<sup>15</sup>

Meanwhile we determined that accurate and reproducible measurements of embryonic brain trajectories (diencephalon, mesencephalon and telencephalon) and embryonic hand measurements can be performed by using transvaginal 3D-US visualizations.<sup>25,26</sup> Size charts of human embryonic brain structures are therefore created and can be used to study normal and abnormal brain development. Measurement of first-trimester trophoblast volumes also shows to be reproducible. Trophoblast volume



**Figure 2.** Illustration of the association between periconceptional maternal conditions and lifestyle and human embryonic growth trajectories<sup>14</sup> (with permission from the Oxford University Press publication and the European Society of Human Reproduction and Embryology, 2015). Fully adjusted model for maternal age (A), periconception alcohol use (B) and smoking (C) (adjusted for gestational age, maternal age, alcohol, smoking, fetal gender and parity) after retransformation to the original scale in millimetres.

and growth in pregnancies ending in miscarriage were found to be significantly smaller compared with pregnancies resulting in live births.<sup>27</sup>

### What are the main strengths and weaknesses?

The main strength of the Predict Study is its unique periconceptional prospective study design, in which research is embedded in standard patient care. Further advantages of this study are the collection of unique and high quality first-trimester exposure data and measurements (questionnaire data, biomarkers, serial 3D imaging data) including unique and detailed embryonic measurements using virtual reality techniques, birth- and placental weight, quantitative data of tissue-specific DNA methylation of genes, biobanking of blood, and DNA and RNA of couples and the infant at fixed study moments. The study also has the ability to elucidate associations as well as underlying molecular biological and epigenetic mechanisms. Furthermore, due to assessing changes in exposure, such as folic acid supplement use and smoking during follow-up, potential bias is reduced. The interdisciplinary collaboration between recognized research groups dedicated to embryonic imaging, (epi)genomics and nutrition is another strength. Furthermore, bioinformatics and novel statistics will be applied in order to perform integrated analysis of imaging, biochemical, dietary and molecular biological and (epi)genetic data. Therefore, we assume that the Predict Study will contribute to the further understanding of the epigenome as a memory of previous exposures and as a predictor of reproductive and pregnancy outcomes. The main weakness of the study is the limited external validation and selection bias due to the enrolment of a relatively

high proportion of high-risk pregnancies. Therefore, the results cannot be solely extrapolated to the general population. Since we only started to collect cord blood from June 2011 onwards, limited cord blood samples are available. We expect to increase these numbers as cord blood sampling will become part of routine procedures in the near future.

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

The Predict Study welcomes colleagues interested in collaboration. In brief, we request a short research proposal including information on the background, research questions and methods, a plan for publication and authorships, timetable and budget. Approval of the proposal depends upon the topic and the quality of the proposal. Furthermore, collaborators have to contribute to the infrastructure for the ongoing general data collection. This can comprise the appointment of a PhD student as well as financial compensation for using data and materials from the Predict Study.

Collaborations are established through a formal contract, which includes mutual obligations (data delivery, rules for publication and presentation, contribution to the Predict Study infrastructure, etc). For more information contact the principal investigators at [predictstudie@erasmusmc.nl].

#### Predict Study in a nutshell

- The Predict Study is an observational prospective open cohort conducted at a tertiary hospital investigating periconceptional influences on reproductive

failures and adverse pregnancy outcomes and health of the offspring up to 1 year of age.

- A total of 792 pregnancies from preconceptionally recruited couples in the Rotterdam area, The Netherlands, were included in 2010–15.
- Follow-up has included seven questionnaires (first trimester to 1 year after delivery), one clinical and three serial ultrasound assessment visits (8–13 weeks of gestation) and an umbilical cord blood sample, with 336 remaining couples eligible for follow-up in addition to the couples to be recruited after 2015.
- The dataset comprises a wide range of phenotypic, nutrition, lifestyle and health measures, biological samples and linkage to health and administrative records.
- The Predict Study can be contacted for collaborations: [predictstudie@erasmusmc.nl].

## Supplementary Data

Supplementary data are available at *IJE* online.

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**Conflict of interest:** None declared.

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