Periconception maternal characteristics and embryonic growth trajectories: the Rotterdam Predict study

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STUDY QUESTION: Are maternal characteristics and lifestyle factors associated with human embryonic growth trajectories?

SUMMARY ANSWER: Periconception maternal age is associated with increased, and smoking and alcohol use with decreased embryonic growth trajectories, estimated with crown-rump length (CRL) measurements.

WHAT IS KNOWN ALREADY: Fetal weight is associated with health and disease in later life. Maternal characteristics and lifestyle factors affect fetal growth in the second and third trimesters of pregnancy and at birth; however, little is known about the association of these characteristics with first trimester embryonic growth.

STUDY DESIGN, SIZE, DURATION: In a tertiary centre, pregnant women were recruited and enrolled in a prospective periconception cohort study before 8 weeks of gestation. We selected 87 spontaneously conceived singleton pregnancies of women recruited in 2009 and 2010 that ended in non-malformed live births.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We performed weekly three-dimensional ultrasound scans from enrolment up to 13 weeks of gestation. At enrolment, a questionnaire was completed. Embryonic CRL measurements were performed using the V-Scope software in the BARCO I-Space. Associations between maternal characteristics and embryonic growth were assessed using square root transformed CRL as response in linear mixed model analyses, adjusted for potential confounders.

MAIN RESULTS AND THE ROLE OF CHANCE: Four hundred and ninety-six scans from 87 pregnancies were included. In the multivariable analysis, maternal age was positively associated with first trimester CRL (difference per maternal year of age 0.024 mm (95% confidence interval (CI) 0.009, 0.040), P = 0.001). At 6 and 12 weeks of gestation, the CRL of an embryo from a 40-year-old mother was estimated 2.0 mm (61%) and 7.2 mm (14%) larger, respectively, compared with an embryo from a 20-year-old mother. Smoking of 10 or more cigarettes per day was negatively associated with CRL (difference 2 0.211 mm (95% CI 2 0.416, 2 0.006), P = 0.04), with embryos that were 0.9 mm (18.7%) and 3.1 mm (5.5%) smaller at 6 and 12 weeks, respectively, compared with non-smokers. Periconception alcohol use was negatively associated with CRL growth rate (difference 2 0.0025 mm/day (95% CI 2 0.0047, 2 0.0003), P = 0.022), with embryos that were 0.2 mm (3%) and 1.1 mm (2%) smaller at 6 and 12 weeks, respectively, compared with non-alcohol users. Parity, BMI and moment of initiation of folic acid use were not significantly associated with embryonic CRL.

LIMITATIONS, REASONS FOR CAUTION: Due to the selection of pregnancies in a tertiary centre and the small number of pregnancies, the external validity of the results has to be confirmed using larger sample sizes and other population-based periconception cohort studies.
WIDER IMPLICATIONS OF THE FINDINGS: The association of maternal age and smoking with embryonic growth is in line with previous literature, whereas the association between embryonic growth and alcohol use is a new finding. However, concerning exposure to alcohol, the effect estimate was small and it is questionable whether this is of clinical value. More research is warranted to unravel underlying mechanisms and to assess the implications for preconception and early pregnancy care, such as the development and implementation of effective lifestyle interventions.

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Key words: crown-rump length / embryonic development / three-dimensional imaging / pregnancy / first trimester

Introduction
High embryonic growth and development rate in the first trimester renders this one of the most vulnerable periods in life. However, until recently, prenatal care and research have focused predominantly on fetal growth in the second half of pregnancy and birthweight. Fetal and newborn weight is associated with health and disease in later life (Gluckman et al., 2009). More recently, embryonic crown-rump length (CRL) at the end of the first trimester has been associated with fetal growth, birthweight and the risk of being born small for gestational age (SGA) or with a low birthweight (Smith et al., 1998; Bukowski et al., 2007; Mook-Kanamori et al., 2010).

During pregnancy the mother is the environment of the developing embryo and fetus (Steegers-Theunissen and Steegers, 2003). Whereas a multitude of maternal characteristics and lifestyle factors, such as parity and smoking, have been linked to birthweight (Gardosi et al., 1992; Wang et al., 2002), few studies have focused on the influence of periconception maternal characteristics and lifestyle on first trimester embryonic size (Bottomley et al., 2009; Bakker et al., 2010; Mook-Kanamori et al., 2010; Prabhu et al., 2010; Sarris et al., 2010). Moreover, to date longitudinal data on embryonic growth remain scarce (Bottomley and Bourne, 2009).

As a result of the development of transvaginal three-dimensional (3D) ultrasound techniques over the last few decades, visualization of the first trimester embryo has improved tremendously. The use of these novel ultrasound techniques in combination with the virtual reality technology of the BARCO I-Space and V-scope visualization software enables depth perception and thus an actual view of the third dimension (Koning et al., 2008). More recently, embryonic crown-rump length (CRL) at the end of the first trimester has been associated with fetal growth, birthweight and the risk of being born small for gestational age (SGA) or with a low birthweight (Smith et al., 1998; Bukowski et al., 2007; Mook-Kanamori et al., 2010).

The aim of this study is to investigate associations between periconception maternal characteristics and first trimester embryonic growth trajectories using longitudinal CRL measurements.

Materials and Methods
Data for this study were collected in the Rotterdam Predict study, an ongoing prospective periconception cohort study that is part of the preconception and antenatal care at the outpatient clinics of Obstetrics and Gynaecology of the Erasmus MC, University Medical Centre Rotterdam, the Netherlands. At enrolment, all participants signed an informed consent form.

All women who were at least 18 years old with ongoing intrauterine singleton pregnancies of 6–8 weeks of gestation were eligible for participation and recruited in 2009 and 2010. The majority of participating women were recruited from the outpatient clinics and one group (25%) was recruited from outside the hospital after hearing of the study from midwives and Erasmus MC staff. Women were informed about the study through brochures and posters, available throughout the outpatient clinics of Obstetrics and Reproductive Medicine, and actively had to contact our study group to sign up for participation.

For the current study we selected only spontaneously conceived pregnancies, including pregnancies conceived after intrauterine insemination (IUI).

Ultrasound data
Women received weekly transvaginal 3D ultrasound scans from enrolment up to the 13th week of pregnancy. Scans were generally performed every 7 days; however, for logistic reasons the number of days between ultrasounds occasionally varied from 6 to 8 days, or 13 to 15 days when women missed an appointment. Ultrasound scans were performed with a 6–12 MHz transvaginal probe using GE Voluson E8 equipment and 4D View software (General Electrics Medical Systems, Zipf, Austria). Afterwards the obtained 3D-datasets were transformed to Cartesian (rectangular) volumes and transferred to the BARCO I-Space (Barco N.V., Kortrijk, Belgium) at the Department of Bioinformatics, Erasmus MC, University Medical Centre Rotterdam. This is a four-walled CAVE™-like (Cave Automatic Virtual Environment) virtual reality system, allowing depth perception and interaction with the projected images (Cruz-Neira et al., 1993). CRL measurements were performed offline using the I-Space and V-Scope software (Koning et al., 2009), and by placing the callipers at the outer side of crown and rump in the mid-sagittal plane. CRL measurements performed in the I-Space show good agreement with two-dimensional measurements (intraclass correlation coefficient (ICC) 0.997 (95% confidence interval (CI) 0.994–0.999)) and very good inter- and intraobserver agreement (both ICCs 1.000 (95% CI 0.999–1.000)) (Verwoerd-Dikkeboom et al., 2008). Every CRL measurement was performed three times by the same researcher, and the mean of these three measurements was used in the analyses.

Questionnaires
At enrolment participants completed a self-administered general questionnaire covering details on maternal age, anthropometrics, ethnicity, education, obstetric history and periconception lifestyle. We define the preconception period as a timespan of 14 weeks before conception.
up to the time of conception. Periconception smoking and alcohol use was defined as smoking any number of cigarettes or drinking any alcoholic beverages up to the moment of pregnancy recognition. The moment of initiation of folic acid supplement use was defined as the initiation at any moment before (pre) or after (post) conception.

Pregnancy dating
Data on the first day of the last menstrual period (LMP) and of regularity and duration of the menstrual cycle were obtained in a personal interview by the researcher performing the ultrasound scan at the first visit. We calculated the gestational age from the LMP in spontaneous pregnancies and from the LMP or insemination date plus 14 days in pregnancies conceived through IUI.

Study population
Of the 259 pregnancies enrolled in the Rotterdam Predict study in 2009 and 2010, we excluded 73 IVF/ICSI pregnancies and two pregnancies in which conception mode was missing. From the resulting 184 spontaneously conceived pregnancies we excluded 54 pregnancies because of the following reasons: 32 pregnancies ended in a miscarriage or an ectopic pregnancy, of 12 pregnancies the first day of the LMP was missing or the observed CRL differed by >6 days from the expected CRL according to the Robinson curve, five pregnancies ended in fetal or neonatal demise (spontaneously or after termination) or major congenital anomalies, three pregnancies with missing questionnaires and in only two pregnancies no periconception folic acid supplements were used and were therefore excluded. Finally, of the remaining 130 pregnancies, we selected for the analyses only pregnancies dated on a regular menstrual cycle of 28 ± 3 days, resulting in a total of 87 pregnancies available for the analyses.

Follow-up
Information on the infant’s date of birth, gender, birthweight and presence of one or multiple congenital anomalies was obtained from medical records. Gestational age at birth was calculated from the dating procedure used in the first trimester as described above.

Statistical analysis
Embryonic growth data were studied using the weekly CRL measurements from ultrasound images performed between 6^10 and 12\^16 weeks.

Maternal characteristics and lifestyle factors considered were age and BMI (continuous), parity (primiparous or multiparous), moment of initiation of folic acid use (pre- or post-conception) and periconception smoking and alcohol use (yes or no). Smoking was also considered in a dose–response sense in three categories: no cigarettes, 1–9 cigarettes per day, 10 or more cigarettes per day.

To estimate selection bias, we compared the maternal characteristics of included and excluded women with and without a regular menstrual cycle of 28 ± 3 days. Continuous data were tested for normal distribution using Kolmogorov–Smirnov, and compared using Student’s t-test or Mann–Whitney U-test. Categorical data were compared using Chi-square or Fisher’s exact test. Birthweight was additionally compared between groups by taking into account gestational age at birth using linear regression.

For the maternal characteristics that were significant in the univariate analysis, potential confounders were identified using analysis of variance with ethnicity and education as explanatory variables, and by calculating Spearman correlation coefficients for the other maternal characteristics listed in Table 1.

To assess the association between maternal characteristics and embryonic growth trajectories we performed stepwise linear mixed model analyses. Square root transformation of CRL data resulted in linearity with gestational age and a constant variance independent of gestational age and was therefore used in the analysis. However, the square root transformed effect estimates resulting from these analyses are difficult to interpret clinically. Results from the linear mixed models are therefore presented not only as the effect estimates directly resulting from these models in √mm but also as differences at 6 and 12 weeks of gestation, after retransformation to the original scale in millimetres.

Firstly, we performed a univariate analysis for all characteristics with and without time interaction in which we adjusted for gestational age only. In the second, multivariable model, we simultaneously entered all variables that were significant in the univariate analysis and fetal gender. In the final, fully adjusted model we additionally entered covariates that were significantly correlated to the maternal characteristics.

Linear mixed model analyses were performed using PROC MIXED in the SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). All other analyses were performed using the Statistical Package for the Social Sciences version 20 for the Windows software (IBM Corp., Armonk, NY, USA). A P < 0.05 was considered statistically significant.

Ethical approval
This study has been approved by the Central Committee on Research in The Hague and the local Medical Ethical and Institutional Review Board of the Erasmus University Medical Centre in Rotterdam in the Netherlands.

Results
A total of 87 pregnancies were included for the analyses. Maternal and pregnancy characteristics of these pregnancies and pregnancies that were excluded because of an irregular menstrual cycle or conception through IVF/ICSI are shown in Table 1.

The median gestational age of the included pregnancies at enrolment was 6^16 (range 6^10–8^13) weeks, and the median number of visits for ultrasound scanning per pregnancy was 6 (range 4–8). From a total of 541 datasets, 496 (91.7%) were of sufficient quality to perform CRL measurements. We performed a median of 6 (range 3–8) CRL measurements per pregnancy.

Mean maternal age was 32.3 years (standard deviation 4.8) and women predominantly had a high level of education (69.5%) and were of Dutch descent (72.4%). BMI ranged from 19.3 to 38.3 kg/m² in the included group, and from 19.1 to 35.0 kg/m² and 18.6 to 33.0 kg/m² in the excluded irregular cycle and IVF/ICSI groups, respectively. Pregnancy complications occurred in 15 (17.2%) pregnancies. Five pregnancies were conceived using IUI procedures (5.7%) and in one of those hormonal stimulation was used (1.1%).

Compared with the women who were excluded due to a less regular menstrual cycle or conception through IVF/ICSI, included women were less often of Dutch ethnicity (P = 0.01), less often initiated folic acid supplement use preconceptionally, more often smoked and used alcohol in the periconception period and less often experienced a maternal
pregnancy complication (Table I. In Supplementary data, Table SI, data are presented separately for pregnancies in women with irregular menstrual cycles and IVF/ICSI treatment). Other characteristics including birthweight, gestational age at delivery and birthweight for gestational age did not differ between included and excluded pregnancies.

### Univariate analysis

Univariate analyses showed that maternal pre-pregnancy BMI, moment of initiation of folic acid use and parity were not significantly associated with CRL (effect estimates and P-values are provided in Supplementary data, Table SII).

Maternal age was significantly positively associated with CRL in the univariate analysis (Table II). Retransformation to the original scale showed that, compared with an embryo of a 40-year-old mother, the embryo of a 20-year-old mother was 1.8 mm (54.7%) and 6.6 mm (12.8%) smaller at 6 + 0 and 12 + 0 weeks of gestation, respectively.

Overall maternal periconception smoking (yes or no) was not significantly associated with CRL, smoking (bm = 0.159, P = 0.003, 95% CI: −0.332, 0.648).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Included</th>
<th>Excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal (at enrolment)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years (mean ± SD)</td>
<td>32.3 ± 4.8</td>
<td>31.8 ± 4.8</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dutch</td>
<td>63 (72.4)</td>
<td>81 (81.8)</td>
</tr>
<tr>
<td>Other western</td>
<td>5 (5.7)</td>
<td>11 (11.1)</td>
</tr>
<tr>
<td>Non-western</td>
<td>19 (21.8)</td>
<td>7 (7.1)</td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>5 (6.1)</td>
<td>10 (10.3)</td>
</tr>
<tr>
<td>Middle</td>
<td>20 (24.4)</td>
<td>36 (37.1)</td>
</tr>
<tr>
<td>High</td>
<td>57 (69.5)</td>
<td>51 (52.6)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.7 (22.3–25.9)</td>
<td>23.9 (21.6–26.2)</td>
</tr>
<tr>
<td>Primiparous</td>
<td>50 (57.5)</td>
<td>69 (68.3)</td>
</tr>
<tr>
<td>Periconception alcohol use</td>
<td>47 (54.0)</td>
<td>38 (37.6)</td>
</tr>
<tr>
<td>Periconception smoking</td>
<td>21 (24.1)</td>
<td>10 (9.9)</td>
</tr>
<tr>
<td>None</td>
<td>66 (75.9)</td>
<td>91 (90.1)</td>
</tr>
<tr>
<td>1–9 cigarettes/day</td>
<td>8 (9.2)</td>
<td>8 (7.9)</td>
</tr>
<tr>
<td>≥ 10 cigarettes/day</td>
<td>13 (14.9)</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>Preconception initiation of folic acid supplement use</td>
<td>62 (72.1)</td>
<td>88 (87.1)</td>
</tr>
</tbody>
</table>

### Data

Data are presented as median (interquartile range) or n (%) unless otherwise specified.

SGA, small for gestational age.

For comparison of distribution of periconception smoking in categories P = 0.004.

Defined as initiation at any moment before conception.

Comparison of birthweight for gestational age P = 0.931.

Defined as weight under the tenth centile for gestational age, gender and parity according to Dutch reference charts (The Netherlands Perinatal Registry, 2011).
embryo that was exposed to alcohol in the periconception period. Retransformation to the original scale showed that an embryo that was exposed to periconception alcohol use, per day increase in gestational age, compared with the reference group. For alcohol, effect estimates represent the amount of change in square root of CRL per day increase in gestational age, compared with the reference group.

In the combined model, significant terms from the univariate analysis and fetal gender were entered into the model simultaneously. Maternal age, periconception smoking of 10 or more cigarettes per day and periconception alcohol use remained significant predictors in the combined model and the effect estimates and significances were similar to those in the univariate analysis (Table III).

The only significant association between maternal characteristics was observed between maternal age and parity ($r = 0.23, P = 0.03$). For the
Final, fully adjusted model, parity was therefore additionally entered into the model, but this did not substantially alter the effect estimates or significance (Table III).

Figure 1 shows the average regression lines from the final, fully adjusted model for maternal age, and periconception smoking and alcohol use, adjusted for fetal gender, parity and gestational age, after retransformation to the original CRL scale, showing the positive association between embryonic growth and maternal age, and an inverse association with maternal periconception smoking and alcohol use.

Discussion

In this periconception prospective study maternal age was associated with increased, and periconception smoking and alcohol consumption with decreased, embryonic growth trajectories.

One of the main strengths of this study is that we obtained longitudinal ultrasound data from early gestation onwards and performed measurements in triplicate using 3D holograms, offering a high degree of precision and reliability (Verwoerd-Dikkeboom et al., 2008). We also collected data prospectively on periconception characteristics and lifestyle. While in pregnancies conceived through IVF the moment of implantation is known, in spontaneously conceived pregnancies variations in the timing of ovulation, implantation and recollection of the LMP result in a less precise determination of gestational age (Jukic and Wilcox, 2012; Mahendru et al., 2012). We therefore excluded all pregnancies with a discrepancy between observed and expected CRL of >6 days.

As this cohort study is embedded in the preconception and antenatal care of outpatient clinics in a tertiary hospital, the proportion of high-risk pregnancies and pregnancy complications by definition is expected to be higher than in a population-based cohort. In addition, our population contains a relatively high proportion of well-educated women using periconceptional folic acid supplements and therefore the external validity of our data will have to be confirmed in other study populations.

We obtained data on periconception lifestyles at study entry, thereby not taking into account lifestyle changes thereafter. However, all women reported to have stopped drinking alcohol and, with the exception of two women, stopped smoking following pregnancy recognition. Therefore, variation in exposure is limited and unlikely to have substantially influenced the estimated associations. We excluded women with a less regular menstrual cycle, who were younger, more often of Dutch descent and experienced more hypertensive pregnancy complications. Although gestational age at birth and birthweight were not different between included and excluded pregnancies, selection bias cannot be excluded completely. In addition we excluded women who conceived through IVF/ICSI treatment, who less often had a high level of education, were more often primiparous and of Dutch descent and endorsed a healthier lifestyle. Women undergoing IVF/ICSI treatment do, by definition, differ from those who conceive spontaneously in terms of the underlying causes of subfertility and increased awareness of poor lifestyle on reproductive outcome. Although this selection is inherent to our study question, it remains to be elucidated whether similar results are observed in IVF/ICSI pregnancies.

There are no clear numbers available of percentages of women with a strictly regular menstrual cycle of 25–31 days in the Netherlands. Studies in other populations suggest that a menstrual cycle of 25–31 days is observed in ≈50–80% of women, increasing with age, of which the higher percentages include women with variations in cycle length.

Figure 1 Final, 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.
(Chiazze et al., 1968; Munster et al., 1992; Kolstad et al., 1999; Mook-Kanamori et al., 2010). The 66% we observed in our study is in line with these findings.

The strongest association with embryonic growth was observed for maternal age. This positive association is in line with previous cross-sectional studies, although at 12 weeks of gestation the estimated difference between a 20 and a 40-year-old mother of 7.2 mm found here was larger compared with the previous studies where differences were observed of 4.2 mm (Bottomley et al., 2009) and 3.5 mm (Mook-Kanamori et al., 2010). Our larger estimate may be explained by the inclusion of only women with strictly regular menstrual cycles, the use of serial measurements and, in the latter case, also on not taking into account gestational age.

Two large cross-sectional cohort studies showed conflicting results for the association between smoking and embryonic size. Prabhu et al. (2010) observed no association between smoking and CRL at 8–12 weeks of gestation; however, the analyses were not restricted to women with a regular menstrual cycle or stratified by the number of cigarettes smoked per day. In an additional analysis of pregnancies with a certain LMP, similar results were obtained; however, in this analysis gestational age was not taken into account (Prabhu et al., 2010). In contrast to these results, but in agreement with those observed in our study, Mook-Kanamori et al. (2010) demonstrated a 1.0 mm smaller embryonic CRL in smokers, although significance attenuated after adjustment for multiple testing; they observed a dose–response relation with a 1.7 mm smaller CRL in smokers of 10 or more cigarettes per day but results were no longer significant in the multivariable model.

The association between periconception alcohol consumption and embryonic growth has not been observed previously. Mook-Kanamori et al. (2010) did not observe significant associations and although they had a larger sample size, a single CRL measurement was performed in a routine clinical setting at a median of 12 weeks of gestational age.

Interestingly, in our study nearly 90% of women who used periconceptional alcohol reported the consumption of 2 or less units per day, which suggests effects on the embryo even with consumption of limited amounts. Previous studies of alcohol use and prenatal growth in later pregnancy are associated with increased growth (Steegers-Theunissen et al., 2009) and 3.5 mm (Mook-Kanamori et al., 2010) and 4.2 mm (Bottomley et al., 2009) and 3.5 mm (Mook-Kanamori et al., 2010). Our larger estimate may be explained by the inclusion of only women with strictly regular menstrual cycles, the use of serial measurements and, in the latter case, also on not taking into account gestational age.

In conclusion, we have shown in a prospective periconception cohort study that maternal age and periconception smoking and alcohol consumption are associated with embryonic growth trajectories. More research is warranted to unravel underlying mechanisms and to assess the implications for preconception and early pregnancy care, such as the development and implementation of effective lifestyle interventions.

Supplementary data
Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors’ roles
E.M.v.U. analysed the data and wrote the first draft of the manuscript. A.H.J.K. and N.E. supervised the acquisition and measurements of ultrasound data. N.v.d.E.O. and J.J.W. assisted in acquisition of data. P.H.C.E. and S.P.W. assisted in data analysis. E.A.P.S. was responsible for the inclusion of patients and the infrastructure of the study. R.P.M.S.T. initiated and designed the study, supervised all aspects of the study and...
contributed to all versions of the manuscript. All the authors were involved in interpretation of the results and revision of the manuscript and approved the final version.

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**Conflict of interest**

None declared.

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