Human embryonic growth trajectories and associations with fetal growth and birthweight

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STUDY QUESTION: How do human embryonic growth trajectories evolve in the first trimester, and is first-trimester embryonic growth associated with fetal growth and birthweight (BW)?

SUMMARY ANSWER: Human embryonic growth rates increase between 9 and 10 weeks of gestation and are associated with mid-pregnancy fetal growth and BW.

WHAT IS KNOWN ALREADY: Fetal growth is associated with health and disease risks in later life. Until recently, prenatal care and research have been focused predominantly on fetal growth in the second and third trimesters of pregnancy. Longitudinal first-trimester data remain scarce.

STUDY DESIGN, SIZE, DURATION: We recruited 201 pregnancies before 8 weeks of gestation in a prospective periconception cohort study conducted in a tertiary center.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We performed weekly 3D ultrasound scans from enrollment up to 13 weeks of gestation. To create embryonic growth trajectories, serial crown–rump length (CRL) measurements were performed using the V-Scope software in the BARCO I-Space. Mid-pregnancy fetal growth parameters and BW were obtained from medical records. Z-scores were calculated for CRL, mid-pregnancy estimated fetal weight (EFW) and BW. Associations between embryonic and fetal growth parameters were investigated using Pearson’s correlation coefficients.

MAIN RESULTS AND THE ROLE OF CHANCE: During the early first trimester (up to 9 weeks of gestation), we observed a constant absolute mean embryonic CRL growth rate of 0.99 mm/day (SD 0.10), while the relative growth rate decreased. Between 9 and 10 weeks of gestation, the absolute growth rate increased, and during late first trimester (from 10 weeks of gestation onward), we observed a constant mean relative growth rate of 4.1% (SD 0.006) per day. Overall, early and late first-trimester median CRL Z-scores were strongly correlated with mid-pregnancy EFW (roverall/early/late = 0.57/0.57/0.54, P < 0.001) but only overall and late CRL Z-scores were correlated with BW (roverall = 0.15, P = 0.04; rearly = 0.10, P = 0.17; rlate = 0.17, P = 0.02).

LIMITATIONS, REASONS FOR CAUTION: This study was conducted in a tertiary hospital. Therefore, future studies in other populations are warranted to confirm our results.

WIDER IMPLICATIONS OF THE FINDINGS: This study shows differences between early and late first-trimester embryonic growth coinciding with changes in intrauterine nourishment. The established associations between first-trimester embryonic growth and fetal size in mid-pregnancy and at birth emphasize that more research is warranted to establish the importance of these results for preconceptional and early pregnancy care.
Introduction

The Barker hypothesis states that intrauterine conditions can affect fetal and newborn weight and subsequent disease risks in later life (Barker, 2007; Gluckman et al., 2008). Although the first trimester of pregnancy is critical for the growth and development of major embryonic organ systems and the placenta (Larsen, 2001), ~20–25% of native Dutch pregnant women in the Netherlands and 18% of pregnant women in the USA (range across different states: 17–31%) do not enter obstetric care before 14 and 12 weeks of gestation, respectively (Center for Disease Control and Prevention, 1999; Bonsel et al., 2010). Until recently, prenatal care and research have been focused predominantly on fetal growth in the second and third trimesters of pregnancy. The high growth rates in the first trimester render this one of the most vulnerable periods in life, in which poor health conditions and lifestyles may have permanent consequences for fetal and post-natal growth, development and health. This is well illustrated by the study of Mook-Kanamori et al. (2010), who demonstrated an inverse association between late first-trimester embryonic size, measured as crown–rump length (CRL), and the risk of adverse birth outcomes. This study also showed that maternal smoking and no use of folic acid supplements are associated with a smaller late first-trimester CRL (Mook-Kanamori et al., 2010). Although this first-trimester cross-sectional data are fascinating, longitudinal data on first trimester embryonic growth remain scarce (Bottomley and Bourne, 2009).

As a result of recent developments of transvaginal 3D ultrasound techniques, visualization of the embryo during the first trimester has improved tremendously. The combination of these novel ultrasound techniques with the virtual reality technology of the BARCO I-Space and V-scope visualization software leads to the ultimate benefit of the third dimension by enabling depth perception and thus an actual view of the third dimension (Koning et al., 2009). Together, these technological developments have enabled the performance of very precise and reliable early first-trimester embryonic measurements in vivo (Verwoerd-Dikkeboom et al., 2008, 2010; Rousian et al., 2010) and have improved the means to assess embryonic growth longitudinally from the early first trimester of pregnancy onward.

The aim of this study is to investigate first-trimester embryonic growth trajectories using longitudinal CRL measurements, and associations between embryonic growth in early and late first trimesters and fetal size in mid-pregnancy and at birth.

Methods

Data for this study were collected in a prospective periconception cohort study at the Department of Obstetrics and Gynaecology at the Erasmus MC, University Medical Centre Rotterdam, the Netherlands. At enrollment, all participants signed a written informed consent form.

All women of at least 18 years old with ongoing singleton pregnancies of 6–8 weeks of gestation were eligible for participation during 2009 and 2010. The majority of participating women were derived from the outpatient clinic of the Department of Obstetrics and Gynaecology at the Erasmus MC, and a small group (25%) was derived from outside the hospital. The latter group heard of the study from midwives and Erasmus MC staff. Women were informed about the study through study brochures and posters, available throughout the outpatient clinics of Obstetrics and Fertility, and actively had to contact the research team to sign up for participation.

Ultrasound data

Women received weekly transvaginal 3D ultrasound scans from enrollment 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 the GE Voluson E8 equipment and the 4D View software (General Electric Medical Systems, Zipf, Australia). Afterward, the obtained 3D data sets 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 calipers at the outer side of crown and rump in the mid-sagittal plane. CRL measurements performed in the I-Space show good agreement with 2D measurements and good inter- and intra-observer agreement (Verwoerd-Dikkeboom et al., 2008). All CRL measurements were performed three times by the same researcher, and the mean of these three measurements was used in the analyses.

Questionnaire data

At enrollment, participants completed a self-administered general questionnaire covering details on maternal age, anthropometrics, ethnicity, education, obstetric history and periconception exposures.

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 at the first visit. We calculated the gestational age from the LMP in spontaneously conceived pregnancies, from the date of oocyte retrieval 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 intrauterine insemination, and from the day of embryo transfer plus 17 or 18 days in pregnancies originating from the transfer of cryopreserved embryos, depending on the number of days between oocyte retrieval and cryopreservation of the embryo. When the menstrual cycle was regular but >3 days different

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from 28 days, we adjusted the gestational age for the duration of the menstrual cycle.

**Study population**

If the first day of the LMP was missing in spontaneously conceived pregnancies, or if the observed CRL differed by >6 days from the expected CRL according to the Robinson curve (Robinson and Fleming, 1975), pregnancies were excluded from the analysis. Furthermore, we selected spontaneously conceived pregnancies and pregnancies conceived through assisted reproductive techniques using biological oocytes from the participating mother-to-be only. In addition, ectopic pregnancies and pregnancies that ended in a miscarriage before 16 weeks of gestation were excluded.

**Follow-up**

In the Netherlands, all pregnant women are offered a routine structural ultrasound examination at ~20 weeks of gestation. Biplanar diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL) measurements performed during this ultrasound examination were retrieved from the medical records. Mid-pregnancy estimated fetal weight (EFW) was calculated using the Hadlock formula: \[
\text{log}_{10}\text{EFW} = 1.326 - 0.00326(\text{AC})(\text{FL}) + 0.0107(\text{HC}) + 0.0438(\text{AC}) + 0.158(\text{FL})
\] (Hadlock et al., 1985).

Information on the infant’s date of birth, gender, birthweight (BW) and the presence of one or multiple congenital anomalies were obtained from medical records. One pregnancy was terminated at 14 weeks after diagnosis of trisomy 21, and, therefore, no birth record was completed.

Gestational age at the time of mid-pregnancy ultrasound examination and at birth was calculated from the dating procedure used in the first trimester, as described above.

**Statistical analysis**

First-trimester embryonic growth trajectories and embryonic growth rates were studied using CRL, absolute CRL growth rate per day and relative CRL growth rate per day of measurements performed between 6+0 and 12+6 weeks. CRL growth rate per day was calculated for the longest strain of consecutive measurements no more than 7 days apart using the following formula: \[
\text{CRL}_{i} - \text{CRL}_{i-1} / \left( \text{GA}_{i} - \text{GA}_{i-1} \right)
\] with CRL representing the CRL at the ith visit in this pregnancy, GA the gestational age at that time and \( \text{GA}_{i} - \text{GA}_{i-1} \leq 7 \) days. The gestational age for the value thus obtained was calculated as the mean of the gestational ages at which the two measurements were performed: \( \frac{\text{GA}_{i} + \text{GA}_{i-1}}{2} \). In pregnancies with two strains of an equal number of consecutive measurements, the strain with the largest gestational age was included. Relative CRL growth rate was calculated as the CRL growth rate divided by the mean of the two CRL measurements from which the absolute growth rate was calculated. Group means were calculated using the median from all gestational ages per pregnancy.

We constructed gestational age-adjusted Z-scores for CRL, mid-pregnancy EFW and BW based on our own data using the R software (version 2.15.1, GAMLSS package version 4.5.1; a more detailed description of the Z-score calculations is provided in the Supplementary data). Z-scores for mid-pregnancy ultrasound data were calculated for all structural ultrasounds performed between 18 and 22 weeks of gestation. Because of a small number of preterm births (\( n = 16 \)), we calculated BW Z-scores for term births only (gestational age \( \geq 37 \) weeks).

Analyses were performed in the total group and in the subgroup of IVF/ICSI pregnancies. In addition, we repeated the analyses in subgroups of: (i) pregnancies with a reliable gestational age based on a very strictly regular menstrual cycle of 28 ± 3 days and a certain LMP or conception date, (ii) spontaneously conceived pregnancies and (iii) uncomplicated pregnancies. The subgroup of complicated pregnancies in our study population was too small and heterogeneous and, therefore, not investigated separately.

To assess associations between embryonic growth and fetal growth parameters, and BW, we used the medians of all the available first-trimester CRL Z-scores per pregnancy.

We used Pearson’s correlation coefficients to assess the associations between CRL and fetal growth parameters, all expressed as Z-scores. All analyses were performed using IBM SPSS Statistics Version 20.0 for Windows (IBM, Armonk, NY, USA).

**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 MC.

**Results**

Of 259 enrolled pregnancies, we excluded 2 pregnancies conceived by oocyte donation, 44 pregnancies that ended in a miscarriage or ectopic pregnancy and 12 pregnancies dated by CRL, resulting in 201 pregnancies available for the analysis of first-trimester embryonic growth trajectories.

The median gestational age at enrollment was 6+0 (range 6+0–6+6) weeks, and the median number of ultrasound visits per pregnancy was 6 (range 4–8). From a total of 1262 data sets, 1144 (90.6%) were of sufficient quality to perform CRL measurements. We performed a median of 6 (range 1–8) CRL measurements per pregnancy.

Routine mid-pregnancy ultrasound data could be obtained for 177 pregnancies (88.1%), and fetal parameters were measured in 86.1–88.1% of pregnancies (BPD: 173 (86.1%); FL: 175 (87.1%); HC and AC: 177 (88.1%). EFW could be computed for 175 (87.1%) pregnancies. Birth records were obtained for 200 (99.5%) pregnancies and BW Z-scores were calculated for all 184 (91.5%) pregnancies with term deliveries.

Maternal and pregnancy characteristics are shown in Table 1. Mean maternal age was 32.2 (SD 4.8) years, and women mainly had a high education (55.2%) and were of Dutch descent (76.8%). In 157 (78.1%) pregnancies, gestational age was based on a strictly regular menstrual period of 28 ± 3 days or conception date, including 61 (30.3% of 201 included pregnancies) pregnancies that were conceived after IVF/ICSI treatment. Pregnancy complications occurred in 43 (21.4%) pregnancies.

**First-trimester embryonic growth trajectories**

In Fig. 1, CRL growth trajectories are depicted for the total group and for IVF/ICSI pregnancies. Growth trajectories demonstrated a smooth curve, although the distribution in the total group was wider than in IVF/ICSI pregnancies only. Growth trajectories in spontaneously conceived pregnancies and in those with a reliable gestational age were comparable with the total group (data not shown).

In 177 (88.1%) pregnancies of the total group and 57 (93.4%) of the IVF/ICSI pregnancies, at least two CRL measurements had been performed no more than 7 days apart, and, in both groups, a median of 3 (range 2–6) embryonic CRL growth rates could be computed. Figure 2 displays the mean absolute and relative growth rates for the total group and for IVF/ICSI pregnancies only. In both groups,
the mean absolute growth rate was constant up to 9 weeks of gestation at 0.99 (SD 0.10) and 1.01 (SD 0.09) mm/day (computed from \( n = 112 \) and \( n = 34 \) pregnancies with embryonic growth rate measurements up to 9 weeks, respectively). After 9 weeks of gestation, the embryonic growth displayed a substantial increase in growth rate, the first onset of which varied approximately between 9 and 10 weeks of gestation. After 10 weeks of gestation, the steady increase in the absolute growth rate translated to a constant mean relative growth rate of 4.1% (SD 0.006) and 3.9% (SD 0.004) per day in spontaneously conceived and IVF/ICSI pregnancies, respectively (computed from \( n = 146 \) and \( n = 46 \) pregnancies with embryonic growth rate measurements from 10 weeks onward). Mean absolute and relative growth rates in subgroups of pregnancies with a reliable gestational age and in spontaneously conceived pregnancies were comparable to those observed in the total group (data not shown).

### Associations between first-trimester embryonic growth and subsequent fetal growth

In Fig. 3, associations between median first-trimester CRL and mid-pregnancy EFW and BW Z-scores are presented for the total group and for IVF/ICSI pregnancies. In the total group, median CRL Z-score was significantly correlated with all fetal growth parameters including mid-pregnancy EFW Z-score (EFW: \( r = 0.57 \), HC: \( r = 0.58 \), BPD: \( r = 0.41 \), AC: \( r = 0.50 \), FL: \( r = 0.41 \); all \( P \)-values < 0.001) and BW Z-score (\( r = 0.15 \), \( P = 0.04 \)), explaining about 33 and 2.3% of the variance, respectively. In IVF/ICSI pregnancies, the estimates were attenuated for mid-pregnancy parameters (EFW: \( r = 0.45 \), \( P = 0.001 \); HC: \( r = 0.28 \), \( P = 0.04 \); BPD: \( r = 0.16 \), \( P = 0.26 \); AC: \( r = 0.42 \), \( P = 0.001 \); FL: \( r = 0.25 \), \( P = 0.06 \)) but became stronger for BW Z-score (\( r = 0.35 \), \( P = 0.008 \)). The explained variance was 20% for EFW and 12% for BW.

We repeated the analyses after stratification for early and late first-trimester CRL. Because of the variation in the timing of the onset of the increase in embryonic growth rate as described before, we defined early trimester as the period up to and including 9 weeks of gestation, and late first trimester as the period from 10 up to 13 weeks gestation. Early and late first-trimester median CRL Z-scores were calculated as the median of all measurements up to and including 9 weeks and from 10 weeks of gestation onward, respectively. In the total group, associations with mid-pregnancy fetal growth parameters were comparable for both early and late CRL with those established with overall first-trimester CRL. However, only late first-trimester CRL was correlated with BW \(( r_{late} = 0.17 \), \( P = 0.02 \); \( r_{early} = 0.10 \), \( P = 0.17 \)), explaining 2.9% of the variance.

In IVF/ICSI pregnancies, early and late first-trimester CRL remained correlated with mid-pregnancy EFW \(( r_{early} = 0.37 \), \( P = 0.008 \); \( r_{late} = 0.28 \), \( P = 0.04 \)) with explained variances of 14 and 8%. The correlations between late CRL and AC was attenuated (AC: \( r_{late} = 0.22 \), \( P = 0.12 \), whereas a correlation between early CRL and BPD emerged (BPD: \( r_{early} = 0.28 \), \( P = 0.05 \)). The early median CRL Z-score was not significantly correlated with BW, whereas the late median CRL Z-score was significantly correlated with BW \(( r = 0.33 \), \( P = 0.01 \)), explaining 11% of the variance.

### Subgroup analyses

The analysis of associations between first-trimester embryonic growth and fetal growth was repeated in a subgroup of 157 pregnancies with a reliable age based on a very strictly regular menstrual cycle of 28 ± 3 days, 140 spontaneously conceived pregnancies and 158 uncomplicated pregnancies. Pearson’s correlation coefficients for all these subgroups and growth parameters are provided in the Supplementary data, Table SI.

In the three subgroups, the correlation coefficients for overall, early and late first-trimester CRL and mid-pregnancy fetal growth

| Table I General characteristics of study population (n = 201). |
|------------------|------------------|
| **Characteristics** | **n** | **Missing** |
| Maternal (at enrollment) \(^a\) | | |
| Age, years | 32.2 ± 4.8 | 9 |
| Ethnicity | | 7 |
| Dutch | 149 (76.8) | |
| Other Western | 16 (8.2) | |
| Non-Western | 29 (14.9) | |
| Education | | 16 |
| Low | 18 (9.7) | |
| Middle | 56 (30.3) | |
| High | 111 (55.2) | |
| BMI (median (range)), kg/m² | 23.8 (18.6–48.9) | 6 |
| Nulliparous | 122 (62.2) | 5 |
| Periconception smoking | 31 (15.8) | 5 |
| Pregnancy and outcome | | |
| Conception through IVF/ICSI | 61 (30.3) | 0 |
| Reliable gestational age \(^b\) | 157 (78.1) | 0 |
| Infant gender, male | 95 (47.5) | 1 |
| BW, g | 3276 ± 636 | 1 |
| Gestational age at delivery (median (range)), week \(^c\) | 39\(1^{+3} \) (14\(3^{+3}–42^{+6}\)) | 1 |
| Pregnancy complications | 43 (21.4) | 0 |
| Maternal | | 17 (8.5) |
| Hypertensive disorder | 14 (7.0) | 1 |
| Gestational diabetes | 4 (2.0) | 1 |
| Fetal | | 28 (13.9) |
| Major congenital anomaly | 6 (3.0) | 1 |
| Fetal/neonatal death | 5 (2.5) | 0 |
| Low BW (<2500 g) | 15 (7.5) | 1 |
| Premature delivery (before 37 weeks) | 16 (8.0) | 1 |
| SGA (<10th customized centile) \(^d\) | 12 (6.3) | 9 |

Data are presented as mean ± SD or n (%), except otherwise specified. BMI, body mass index; IVF/ICSI, in vitro fertilization with or without intra-cytoplasmic sperm injection; BW, birthweight; SGA, small for gestational age.

\(^a\)For five women, all maternal characteristics are missing because of unreturned questionnaires.

\(^b\)Gestational age based on a menstrual cycle of 28 ± 3 days or conception date.

\(^c\)Defined as weight under the 10th centile for gestational age, gender and parity according to Dutch reference charts (The Netherlands Perinatal Registry, 2011).
parameters and BW were of similar size and significance to those observed in the total group. However, exceptions were pregnancies with a reliable gestational age, in which the correlation between CRL and BW emerged in early first trimester ($r_{\text{early}} = 0.18$, $P = 0.04$) and became stronger in late first trimester ($r_{\text{late}} = 0.25$, $P = 0.003$), and in spontaneously conceived pregnancies in which both early and late median CRL $Z$-scores were not significantly correlated with BW.

**Discussion**

In this prospective study, from early pregnancy onward we have observed that the first-trimester embryonic growth rate is approximately constant up to 9 weeks of gestation and substantially increases after 9 to 10 weeks of gestation. Furthermore, first-trimester embryonic growth appears to be strongly correlated with mid-pregnancy fetal growth parameters and less strongly with BW. Correlations with BW were stronger and more consistently observed for late than for early first-trimester embryonic growth. Finally, associations were comparable in pregnancies with the most reliable pregnancy dating and in IVF/ICSI pregnancies.

This study has several strengths. We acquired weekly ultrasound data from the earliest stages of pregnancy up to 13 weeks of gestation in more than 200 pregnancies. Furthermore, we performed all CRL measurements using true 3D holograms, offering a high degree of precision and reliability (Verwoerd-Dikkeboom et al., 2008), which is even further increased by using the mean of three CRL measurements per time point per pregnancy for the analyses. In addition, fetal growth data from mid-pregnancy and birth were obtained from medical records rather than from questionnaires. An important issue which we considered in the design and analysis of the study is the dependency of embryonic growth on gestational age. While in IVF/ICSI pregnancies the moment of implantation is the only determinant of gestational age, in spontaneously conceived pregnancies variations in timing of ovulation and implantation and recollection of LMP result in a less precise determination of gestational age. For that reason, we excluded pregnancies with a discrepancy between observed and expected CRL of $>6$ days. Furthermore, we repeated the analyses in a subgroup of pregnancies with the most reliable gestational age, which did not substantially alter the results. Finally, we stratified the analysis by mode of conception. In all groups, a significant correlation of embryonic growth with mid-pregnancy EFW was observed. In the total group of pregnancies, we showed a correlation between embryonic growth and BW, which was stronger in pregnancies with a reliable gestational age and IVF/ICSI pregnancies, but absent in only spontaneously conceived pregnancies. These data support the internal validity of our results, but also show some confounding by a less precise pregnancy dating in spontaneously conceived pregnancies. In addition, embryonic growth seems not to be uniform but is also influenced by maternal conditions, endometrial receptivity and exposures, such as maternal age, ethnicity and smoking (Bottomley et al., 2009; Mook-Kanamori et al., 2010). Therefore, we dated pregnancies using LMP rather than CRL. Maternal influences on embryonic growth are a very important and interesting issue to be further investigated in large periconception cohort studies in the future.

There are some limitations that have to be addressed as well. This study was carried out in a tertiary hospital and, therefore, its external validity is expected to be limited. The proportion of high-risk pregnancies and pregnancy complications is likely to be higher than in a population-based cohort study. However, after repeating the analyses in a subgroup of uncomplicated pregnancies, the results were comparable with the total group. Because our study population also contains a relatively high proportion of women with a higher education and pregnancies conceived after IVF/ICSI treatment, our results will have to be confirmed in other populations. Unfortunately, the current study population was too small to study the association between first-trimester embryonic growth and adverse pregnancy outcome.

**Figure 1** Longitudinal first-trimester embryonic growth trajectories, measured by weekly CRL measurements for the total group of pregnancies (A) and IVF/ICSI pregnancies only (B).
Individual embryonic growth trajectories displayed a smooth curve and an increasing growth rate in late first trimester. The onset of the observed increase in embryonic growth rate between 9 and 10 weeks of gestation is in line with Deter et al. (1999), who showed an increase in growth rate at 9 + 1 weeks of gestation. This period coincides with the transition from histiotrophic to hemotrophic nutrition. In the early first trimester of pregnancy, nourishment of the embryo is characterized by the transport of carbohydrate-rich proteinaceous secretions from the uterine glands into the intervillous space of the developing placenta, i.e. histiotrophic nutrition (Burton et al., 2001). The secretions are phagocytosed by the trophoblast, and nutrients pass into the celomic cavity, from where they may be transported to the embryo via the yolk sac. Eight to 9 weeks after conception, trophoblast plugs originally blocking the spiral arteries gradually dissipate, and maternal blood begins to enter the marginal zone of the placenta. This leads to a transition from a histiotrophic to an increasingly hemotrophic nutrient and oxygen supply of the embryo and thus the initiation of the hemochorial function of the placenta (Burton et al., 1999, 2001; Merce et al., 2009). As a result, there is a 3-fold increase in the intra-placental oxygen concentration between the end of the first, and the start of the second, trimester. Thus, if the increase in embryonic growth rate occurs as a consequence of this transition, factors influencing dissipation of the plugs may also change the timing of the increase in embryonic growth rate itself. Pregnancy complications such as pre-eclampsia and fetal growth restriction have been associated with premature loosening of these trophoblast plugs, which gives rise to excessive placental oxidative stress (Burton and Jauniaux, 2011).

Figure 2 Mean absolute and relative embryonic CRL growth rates, for the total group (A and B) and IVF/ICSI pregnancies only (C and D).

We demonstrated a strong correlation between embryonic growth and mid-pregnancy fetal growth parameters which is in line with data from a large population-based prospective cohort study showing correlations of similar magnitude between late first-trimester CRL and HC, FL and mid-pregnancy EFW (Mook-Kanamori et al., 2010). Although the observed estimates were small, we were able to demonstrate an association between first-trimester embryonic growth and BW. The presence of this association despite the time interval of ~7 months, and the fact that most fetal weight gain occurs in the last trimester of pregnancy, stresses the importance of pre- and periconception maternal conditions, lifestyles and care. One of the potential mechanisms underlying the association between first-trimester growth and BW is the programming of the embryonic genome by epigenetic mechanisms with consequences for subsequent fetal growth. This is in line with the developmental origin of health and diseases, in which the prenatal environment of the fetus is an important determinant of future health and disease. The weak association between embryonic growth and BW is supported by the majority of studies conducted in pregnancies conceived through artificially reproductive techniques and spontaneous pregnancies. In pregnancies conceived through artificial reproductive techniques, embryonic CRL has been positively associated with BW and inversely with risk of having a small for gestational age (SGA) infant (Dickey and Gasser, 1993; Bukowski et al., 2007; Salomon et al., 2011). In two large prospective cohorts of spontaneously conceived pregnancies, a small CRL was associated with an increased risk of low BW and SGA.

**Figure 3** Correlations of first-trimester median CRL and mid-pregnancy EFW and BW Z-scores for the total group (A: Pearson’s correlation coefficient \( r = 0.57, r^2 = 0.33, P < 0.001 \); and B: \( r = 0.15, r^2 = 0.02, P = 0.04 \)) and IVF/ICSI pregnancies only (C: \( r = 0.45, r^2 = 0.20, P = 0.001 \); and D: \( r = 0.35, r^2 = 0.12, P = 0.008 \)).
(Smith et al., 1998; Mook-Kanamori et al., 2010). In contrast, in a large cohort study, no association was observed between a small CRL and SGA risk (Pedersen et al., 2008). However, in the same study, an association was observed between first-trimester BPD below the 10th centile and increased SGA risk (Pedersen et al., 2008).

To our knowledge, this is the first study to show associations between BW and early and late first-trimester embryonic growth separately. Whereas most studies on late first-trimester CRL support our findings and report positive associations between CRL measurements after 10 weeks of gestation and BW, early CRL was measured from 6–14 weeks onward in only one other study (Dickey and Gasser, 1993). In the analysis, however, all measurements up to 10–16 weeks were included and early embryonic growth was not investigated separately (Dickey and Gasser, 1993).

In conclusion, we have shown in a prospective periconception cohort study that first-trimester embryonic growth trajectories and growth rates show individual variation. First-trimester embryonic growth, particularly from 10 weeks of gestation onward, appears to be associated with fetal and newborn growth parameters, emphasizing the need for more research to establish the implications of these results for preconceptional and early pregnancy care. Further investigation and extension of this cohort will enable an estimation of the onset of the increase in embryonic growth rate in the first trimester, define the extent to which this moment influences pregnancy course and outcome, determine whether it is influenced by periconception constitutional factors and exposures and will ultimately allow assessment of the predictive value of first-trimester CRL for normal and adverse pregnancy outcome.

Supplementary data

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

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Authors’ roles

E.M.U. analyzed 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. P.H.C.E. and S.P.W. assisted in data analysis. J.S.E.L. and E.A.P.S. were responsible for the included patients and the infrastructure of the study. G.J.B. significantly contributed to the explanation of the results with regard to his previous work on human intrauterine nutrition. 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 authors were involved in the interpretation of the results and revision of the manuscript and approved the final version.

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Conflict of interest

The authors declare no conflicts of interest.

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