Periconceptional maternal 'high fish and olive oil, low meat' dietary pattern is associated with increased embryonic growth: The Rotterdam Periconceptional Cohort (Predict Study)

Short title: Diet and embryonic growth

Francesca Parisi[1], Melek Rousian[1], Nicole A. Huijgen[1], Anton H.J. Koning[2], Sten P. Willemsen[3], Jeanne H.M. de Vries[4], Irene Cetin[5], Eric A.P. Steegers[1], Régine P.M. Steegers-Theunissen[1, 6*]

[1] Department of Obstetrics and Gynecology, Erasmus MC, University Medical Centre, Rotterdam, the Netherlands

[2] Department of Bioinformatics, Erasmus MC, University Medical Centre, Rotterdam, the Netherlands

[3] Department of Biostatistics, Erasmus MC, University Medical Centre, Rotterdam, the Netherlands

[4] Human Nutrition, Wageningen University, Wageningen, the Netherlands

[5] Center for Fetal Research Giorgio Pardi, Department of Biomedical and Clinical Sciences, Hospital Luigi Sacco, Università degli Studi di Milano, Milan, Italy

[6] Department of Pediatrics, Division of Neonatology, Erasmus MC, University Medical Centre Rotterdam, the Netherlands

*Corresponding author:

Régine P. M. Steegers-Theunissen, MD, PhD, Professor in Periconception Epidemiology

Department of Obstetrics and Gynecology, Erasmus MC, University Medical Centre Rotterdam, PO Box 2040, 3000CA, Rotterdam, The Netherlands.

Tel: 0031-10-7038256; Fax: 0031-10-7036815;

E-mail: r.steegers@erasmusmc.nl

Key words: virtual reality; 3D ultrasound; crown-rump length; embryonic volume; first trimester; maternal dietary patterns; periconceptional period.

Abstract

Objective To investigate associations between periconceptional maternal dietary patterns and first trimester embryonic growth.

Methods 228 women with singleton ongoing pregnancies were enrolled in a prospective periconceptional cohort study, comprising of 135 strictly dated spontaneous pregnancies and 93 pregnancies achieved after *in vitro* fertilization or intracytoplasmatic sperm injection (IVF/ICSI pregnancies). All women underwent longitudinal transvaginal three-dimensional ultrasound (3D-US) scans from 6^{+0} up to 13^{+0} weeks of gestation. Crown-rump length (CRL) and embryonic volume (EV) measurements were performed using a virtual reality system. Periconceptional maternal dietary intakes were collected via food frequency questionnaires (FFQ). Principal component analysis was performed to identify dietary patterns. Associations between dietary patterns and CRL and EV trajectories were investigated using linear mixed models adjusted for potential confounders.

Results A median of five (range 1-7) 3D-US scans per pregnancy were performed. 991 out of 1162 datasets (85.3%) were of sufficient quality to perform CRL measurements and 899 for EV measurements (77.4%). A 'high fish and olive oil, low meat' dietary pattern comprising of high intakes of fish and olive oil, and very low intake of meat was identified. In strictly dated spontaneous pregnancies, a strong adherence to this dietary pattern was associated with a 1.9 mm (95% CI: 0.1, 3.63) increased CRL at 7 weeks (+14.6%) and 3.4 mm (95% CI: 0.2, 7.81) at 11 weeks (+6.9%), whereas EV increased by 0.06 cm^3 (95% CI: 0.01, 0.13) at 7 weeks (+20.4%) and 1.43 cm^3 (95% CI: 0.99, 1.87) at 11 weeks (+14.4%) respectively. No significant associations were observed in the total study population and IVF/ICSI pregnancies.

Conclusions Periconceptional maternal adherence to a ‘high fish and olive oil, low meat’ dietary pattern is positively associated with embryonic growth in strictly dated spontaneous pregnancies.

INTRODUCTION

Maternal nutrition is the main determinant of fetal nutrition known to influence pregnancy outcome as well as future health of the offspring.[1, 2] Nevertheless data are scarce about the influence of periconceptional maternal nutrition on embryonic growth.[3-7] This is related to the fact that in clinical practice the embryonic period is often missed and to the widespread assumption that embryonic growth is the same in every pregnancy and woman.[8] However, in the last decade new insights reveal that first trimester embryonic growth differs and is significantly associated with periconceptional maternal characteristics, nutrition and lifestyle, including age, ethnicity, smoking and alcohol consumption.[9-12] In addition, a curvilinear association was shown between periconceptional maternal folate status and embryonic growth, while strong adherence to an energy-rich dietary pattern significantly increased late first trimester crown-rump length (CRL) measurements in the Generation R study.[13, 14] These data emphasize the need for the development of customized embryonic growth curves. Since most reproductive failures and adverse pregnancy outcomes originate in the periconceptional period (time window: 14 weeks before up to 10 weeks after conception), customized growth curves may serve as early predictors of adverse pregnancy outcome in the future.[15, 16]

Over the last decades, safe, highly precise and reliable measurements of early embryonic structures have been performed using transvaginal three-dimensional ultrasound (3D-US) with high frequency probes and offline visualization in a virtual reality (VR) system.[17] Furthermore, the introduction of the Barco I-Space VR system and the V-Scope software allows automatic and precise embryonic volume (EV) measurements with high intra-observer and inter-observer agreement, thereby providing

EV reference charts.[18] Nowadays principal component analysis (PCA) is a standard statistical analysis which derives dietary patterns from food frequency questionnaires (FFQs) by data-driven dimension reduction techniques, further validated with biomarker concentrations and associated with complex diseases. [19]

The aim of this study is to investigate associations between periconceptional maternal dietary patterns and first trimester embryonic growth, using longitudinal CRL and EV measurements as outcome.

SUBJECTS AND METHODS

This study was embedded in the ongoing Rotterdam Periconceptional Cohort (Predict Study), a prospective hospital-based birth cohort study, conducted at the Department of Obstetrics and Gynecology of the Erasmus MC, University Medical Centre in Rotterdam, the Netherlands.[20] The protocol was approved by the Medical Ethical and Institutional Review Board at the Erasmus MC, University Medical Centre in Rotterdam, the Netherlands, and all participants signed a written informed consent (METC Erasmus MC 2004-277).

Pregnant women of at least 18 years of age were eligible for participation and were recruited before 8^{+0} weeks of gestation between November 2010 and July 2014 (Figure 1). From a total of 400 pregnancies, we excluded: 48 pregnancies complicated by twinning, miscarriage, ectopic implantation, congenital anomalies and intrauterine fetal death; 5 pregnancies conceived after oocyte donation; 26 pregnancies with missing (n=11) or unreliable (n=15) nutritional data. The remaining 321 patients included 93 IVF/ICSI pregnancies derived from *in vitro* fertilization (IVF), intracytoplasmatic sperm injection (ICSI) and cryo-embryo transfer. Among the 228 spontaneously and intrauterine insemination (IUI) conceived pregnancies, we selected women with strict pregnancy dating defined by a known first day of the last menstrual period (LMP), regular cycle and CRL observed < 7 days different from expected according to the Robinson curve (strictly dated spontaneous pregnancies, n=135).[3] The gestational age was calculated from LMP for strictly dated spontaneous pregnancies (with adjustment for cycle duration if <25 or >31 days), from LMP or insemination date plus 14 days for IUI pregnancies, from the day of oocyte retrieval plus 14 days for the IVF/ICSI pregnancies and from embryo transfer day plus 17 or 18 days in pregnancies derived from transfer of cryopreserved embryos. In this way, the total study population included pregnancies with reliable and strict dating by definition. Since an effect of conception mode on embryonic growth and responses to nutritional exposures cannot be excluded, we performed the analysis in the total study population and after stratification in the two subgroups of strictly dated spontaneous and IVF/ICSI pregnancies.

All women received longitudinal transvaginal 3D-US scans from 6^{+0} weeks up to 13^{+0} weeks of gestation with a 6-12 MHz transvaginal probe using GE Voluson E8 equipment and 4D View software (General Electrics Medical Systems, Zipf, Austria). Since the pilot study showed an accurate modeling of embryonic growth curves with three scans per patients, 3D-US scans were generally performed every 7 days between 2010 and December 2012, and reduced to 3 scans per patient (at 7, 9, 11 weeks of gestation) after January 2013.[10] The obtained 3D-US 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, in order to perform offline CRL and EV measurements using the V-Scope software. A length-measuring tool was used to perform length measurements in three dimensions (CRL). A semi-automated volume measuring application based on gray-scale differences was used to perform EV measurements. CRL and EV measurements and reliability have been extensively described elsewhere and excellent inter- and intra-observer agreement has been previously reported.[18, 21] CRL measurements were performed three times by a trained researcher and the average was used in the analysis. EV measurements were performed once in the same image selected for the CRL measurement.

At enrollment all participants filled out a general questionnaire providing details on age, ethnicity, educational level, obstetric and medical history and periconceptional lifestyle (smoking, alcohol use, folic acid or multivitamin supplements use). Anthropometric measurements were obtained by trained counselors (height, weight).

The validated semi-quantitative food frequency questionnaire (FFQ), developed by the division of Human Nutrition, Wageningen University, the Netherlands, and validated for women of reproductive age was used at enrollment to estimate habitual food intake over the previous four weeks.[22, 23] The FFQ consists of 196 food items structured according to meal patterns, including questions on consumption frequency, portion size, and preparation method. Energy and nutritional intakes were determined using the Dutch food composition table (2011).[24] The FFQs were checked in a standardized manner for completeness and consistency. First trimester fasting venous blood samples were collected at enrollment for serum folate and vitamin B12 and plasma total homocysteine (tHcy) assessment. The laboratory procedures have been extensively described elsewhere.[13]

Data on birth outcomes were obtained from medical records (date of birth, gender, birth weight, congenital anomalies). Gestational age at birth was calculated from the dating procedure used in the first trimester as described above.

Statistical analysis

Maternal characteristics were compared between included and excluded pregnancies using Chi-square or exact tests for ordinal variables and Mann-Whitney U test for continuous variables.

PCA was applied to identify dietary patterns in women with reliable FFQs as extensively described by Hoffmann and performed in several studies.[14, 25, 26] We reduced 196 food items from the FFQs to 11 predefined food groups based on origin and similar nutrient content (cereals, olive oil, solid fat, fish, fruit, grain, vegetables, meat, snacks, sugars, alcohol). Since maternal alcohol consumption has been associated with embryonic growth in our previous study, we excluded alcohol from dietary pattern extraction and consider its use as a confounder for further adjustment.[10] Practically, PCA is a standard multivariate statistical technique that aggregates specific food groups on the basis of the degree to which food items are reciprocally correlated. Only dietary patterns (principal components) with eigenvalues ≥1.1 were extracted, in order to reduce bias of multiple testing and to identify the most common dietary patterns in the study population. When PCA is performed, a factor loading is automatically calculated for each food group, showing the extent to which each food group is correlated with the specific dietary pattern. We used three factor loadings with the highest absolute value to label the dietary patterns. Finally, all women automatically receive a factor score for every dietary pattern representing their adherence to that specific dietary pattern. Kruskal-Wallis test was used to compare the adherence to each dietary pattern between included and excluded women. Since the FFQ was validated for the assessment of folate and vitamin B12 intake, maternal biomarkers of one-carbon metabolism, including folate, vitamin B12, and tHcy, were compared between women with strong adherence (positive factor scores) *versus* weak adherence (negative factor scores) to each dietary pattern using Mann-Whitney *U*-tests.[23] Lastly, the food intake level (FIL) was calculated as the ratio of energy intake divided by basal metabolic rate (BMR) and compared with a physical activity level (PAL) of a sedentary lifestyle in order to evaluate underreporting of food intake (new Oxford equations stratified by age).[27]

Linear mixed models, which allow the modelling of longitudinal measurements accounting for the dependent observations within the same pregnancy, were firstly estimated to evaluate associations between conception mode and embryonic growth in the total study population. Square root transformation of CRL data and third root transformation of EV data were performed to obtain a normal distribution of observations as required by linear mixed models and resulted in linearity with gestational age and a constant variance independent from gestational age. Linear mixed models were secondly estimated to evaluate associations between dietary patterns, food groups and embryonic growth in both total study population and strictly dated spontaneous and IVF/ICSI pregnancy subgroups. We performed a crude model using gestational age as predictor and with adjustment for energy intake. In the fully adjusted model, we additionally entered all potential confounders (parity, alcohol use, smoking, folic acid/multivitamin supplement use, maternal age, BMI and comorbidity, fetal gender). Maternal chronic comorbidities considered for adjustment were cardiovascular, autoimmune, metabolic and endocrine diseases.

P-values ≤0.05 were considered significant. All analyses were performed using SPSS Statistics for Windows, Version 21.0 (IBM Corp. Armonk, NY) and R version 3.2.1 (The R Foundation for Statistical Computing).

RESULTS

A total of 228 singleton ongoing pregnancies were included for the analysis, comprising of 135 strictly dated spontaneous pregnancies and 93 IVF/ICSI pregnancies. The median gestational age at recruitment was 7^{+1} weeks and the median number of 3D-US scans per pregnancy was five (range 1-7). From a total of 1162 datasets, 991 were of sufficient quality to perform CRL measurements (85.3%) and 899 to perform EV measurements (77.4%). Baseline characteristics and pregnancy outcomes are listed in Table 1 with comparisons between included and excluded pregnancies.

By using PCA we obtained three uncorrelated dietary patterns explaining 46.8% of the variance of the overall dietary intake of the total study population (Table 2). The first component was labelled 'high vegetables, fruits and grain dietary pattern' (18.5% explained variance). The second component was labelled 'high solid fat, snacks and sugars dietary pattern', also associated with a low intake of fruit (17.4% of the variance). The third component was labelled 'high fish and olive oil, low meat dietary pattern' (10.9% explained variance). No differences in the adherence to the three dietary patterns (factor scores) were observed between included and excluded pregnancies, as well as between strictly dated spontaneous and IVF/ICSI pregnancy subgroups. Women with strong adherence to the 'high vegetables, fruits and grain' dietary pattern (defined by factor scores >0) showed significantly higher vitamin B12 concentrations (median values: 331 pmol/l (range 95-713 pmol/l) *versus* 279.5 pmol/l (range 109-953 pmol/l), p=0.01), as well as lower concentrations of tHcy (median values: 6.0 µmol/l (range 4.0-18.0 µmol/l) *versus* 6.6 µmol/l (range 3.0-14.0 µmol/l), p<0.01) compared to women with weak adherence to the same dietary pattern (factor scores <0). In contrast, women with strong adherence to the 'high solid fat, snacks and sugars' dietary pattern showed a lower serum folate compared to women with weak adherence to the same dietary pattern (median values: 38.4 nmol/l (range 11-187 nmol/l) *versus* 37.0 nmol/l (range 12-118 nmol/l), $p=0.05$). No significant differences in the investigated maternal biomarkers were detected according to the adherence to the 'high fish and olive oil, low meat' dietary pattern.

Linear mixed model analysis showed no significant differences in longitudinal CRL and EV measurements between strictly dated spontaneous and IVF/ICSI pregnancies (fully adjusted model; group effect on CRL analysis: $\beta=0.05$ $\sqrt{mm}$ (95% CI: -0.03, 0.12), $p=0.27$; EV analysis: $\beta=0.02$ $\sqrt[3]{cm^3}$ (95% CI: -0.02, 0.06), $p=0.29$). Table 3 shows the results from linear mixed models. No significant associations were observed between maternal dietary patterns and longitudinal CRL measurements in the total study population and IVF/ICSI pregnancy subgroup. The analysis showed a significant positive association between the 'high fish and olive oil, low meat' dietary pattern and longitudinal CRL measurements in the strictly dated spontaneous pregnancy subgroup, for both crude and fully adjusted models. The transformation to the original scale showed that strong adherence to the 'high fish and olive oil, low meat' dietary pattern (defined as +2 standard deviations (SD) in factor score) increased CRL by 1.9 mm (95% CI: 0.1, 3.63) at 7 weeks (+14.6%) and 3.4 mm (95% CI: 0.2, 7.81) (+6.9%) at 11 weeks compared to weak adherence (-2 SD in factor score). The analysis on EV confirmed no significant results in the total study population and IVF/ICSI pregnancies, while only the 'high fish and olive oil, low meat' dietary pattern was significantly associated with increased longitudinal EV measurements in strictly dated spontaneous pregnancies, in both crude and fully adjusted models. The transformation to the original scale showed that strong adherence (+2 SD in factor score) to this dietary pattern increased EV by 0.06 cm^3 (95% CI: 0.01, 0.13) at 7 weeks (+20.4%) and by 1.43 cm^3 (95% CI: 0.99, 1.87) at 11 weeks (+14.4%) compared to weak adherence (-2 SD in factor score). Figure 2 shows the average regression lines from the fully adjusted model for the 'high fish and olive oil, low meat' dietary pattern in strictly dated spontaneous pregnancies. Finally, linear mixed models showed no associations between the single food groups highly associated with the 'high fish and olive oil, low meat' dietary pattern and longitudinal CRL and EV measurements in the total study population and two subgroups.

DISCUSSION

We showed significant associations between periconceptional maternal adherence to a 'high fish and olive oil, low meat' dietary pattern and increased embryonic growth, depicted by longitudinal CRL and EV measurements, among strictly dated spontaneous pregnancies. Mean CRL measurements were in line with the Robinson curves,[3] while EV measurements were comparable with previous results.[28] Our results point out a greater effect of dietary patterns on EV compared to CRL and in the early compared to the late first trimester embryo. Previous research showed that maternal adherence to an energy rich dietary pattern, resembling our 'high solid fat, snacks and sugars' dietary pattern, increased first trimester CRL.[14] Despite a larger sample size, only a single CRL measurement in a routine clinical setting at 12 gestational weeks was performed.

Fish intake, as omega-3 fatty acid rich food group, has been previously related to improved embryo morphology scores in the IVF population,[29] as well as to higher birth weight, but results are controversial.[30-33] Controversies are probably due to the beneficial effect of omega-3 fatty acids on cell membrane synthesis, gene expression, and eicosanoid metabolism and the simultaneous adverse effect of contaminants, both present in seafood. [32, 34, 35] Our results largely substantiate these findings. Fish intake as a single exposure was not associated with embryonic growth. The effect of a single nutrient is in most cases too small to detect. Moreover, the (un)known interactions and cumulative effects of multiple nutrients included in a dietary pattern are much stronger and therefore can explain our results. The extracted dietary pattern was also related to a very low intake of meat. Recent evidences showed that high processed meat intake is negatively associated with fertilization, implantation and pregnancy rates among couples undergoing conventional IVF.[36,37] Moreover, a dietary pattern high in red meat intake was negatively associated with second and third trimester fetal growth parameters.[38] Recent animal data also showed that maternal olive oil increased piglet birth weight, while reducing plasma IL-1β and TNF-α levels in the offspring.[39] We suggest that the lower intake of saturated fats and the increased omega-3/omega-6 fatty acid ratio, both expected in case of strong adherence to the 'high fish and olive oil, low meat' dietary pattern, could impact embryonic growth possibly by modulating inflammation and oxidative stress pathways.[40,41] Our findings suggest that the focus of caregivers should be on the recommendation of a healthy dietary pattern instead of single healthy food intake to (pre)pregnant women.[42]

We found no significant associations between dietary patterns and embryonic growth in IVF/ICSI pregnancies, which may also explain the non-significant associations in the total study population. We suggest that the IVF/ICSI technique has a stronger effect on embryonic growth than periconceptional maternal dietary patterns, possibly influencing embryonic responses to maternal exposures. Of interest is to address that recent studies demonstrated an independent effect of culture media on birth weight, showing associations with fetal growth starting as early as the second trimester of pregnancy.[43,44] Moreover, when IVF/ICSI is performed, the embryo is not exposed to the natural maternal nutritional environment during the first 3-5 days of development, an essential period when epigenetic reprogramming takes place. [45] This could explain the missing association with dietary patterns. Another explanation, inherent to observational studies and despite the adjustment for many covariates, is that residual confounding cannot be excluded.

The main strength of our study is the longitudinal evaluation of embryonic growth with a median of five scans per patient, the use of a VR system and three independent CRL measurements per time point, providing an accurate picture of first trimester growth process. Finally we performed the automatic EV measurement on the same datasets with high success rates. A retrospective study recently showed that EV represents a more effective measurement of first trimester growth restriction in aneuploidy fetuses compared to CRL, since all three dimensions are taken into account instead of one dimension.[46] We minimized confounding of gestational age by including women with strict pregnancy dating only, based on a known LMP, regular cycle and concordant CRL. This means that all ultrasound measurements could be read as response variables and outcome measurements. In order to reduce selection bias, we compared baseline characteristics of included and excluded women and further adjusted the analysis for multiple maternal covariates, including BMI and age. In the present study, a PCA was used with the advantage to take into account the correlation of food groups without a hypothesis-oriented approach.[47] Moreover, the assessment of biomarkers of one carbon metabolism validates the dietary patterns.[23] Since previous studies demonstrated an overall stability of maternal dietary patterns before and during pregnancy, an early first trimester dietary questionnaire provides a valid representation of maternal diet over the periconceptional period.[48] Moreover, the FFQ was validated for the target group.[23]

Inherent to the observational design of the study, some limitations have to be addressed. The associations between the dietary pattern and embryonic growth are statistically significant, but the clinical relevance of small effect sizes has to be further investigated. Moreover dietary surveys could be prone to bias.[49] We tested underreporting using a PAL cutoff of 1.35 and we estimated a FIL mean value of 1.36 in the included population reducing the likelihood of underreporting. This cohort study is embedded in a tertiary hospital, which means by definition that the proportion of maternal comorbidity and pregnancy complications is expected to be higher than in a population-based cohort, reducing the external validity of our findings. Finally, further investigations including the assessment of maternal fatty acid biomarkers are needed to substantiate our findings.

Previous studies showed that first trimester CRL measurements are strongly associated with subsequent fetal growth parameters, the risk of preterm birth, low birth weight and small for gestational age babies and the cardiovascular risk profile in childhood. [12, 50, 51] All these results underline that first trimester growth is associated with pregnancy outcome and future health of the offspring. Therefore, improving periconceptional maternal modifiable risk factors seems relevant in order to ameliorate pregnancy outcome and future wellbeing of the offspring.

In conclusion, we have shown that a periconceptional maternal 'high fish and olive oil, low meat' dietary pattern is associated with increased embryonic growth in strictly dated spontaneous pregnancies. More research and intervention studies are warranted to investigate the effects in the general population, to reveal underlying mechanisms and to assess the implications for preconceptional and pregnancy care.

ACKNOWLEDGMENTS

We would like to thank the contribution of the Rotterdam Periconceptional Cohort ‘Predict team’ of the Department of Obstetrics and Gynecology at the Erasmus MC, University Medical Centre in Rotterdam, the Netherlands, for recruitment, data collection and cleaning.

REFERENCES

1. Barker DJ. The origins of the developmental origins theory. *J Intern Med* 2007;**261**:412-417.
2. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med* 2008;**359**:61-73.
3. Robinson HP. Sonar measurement of fetal crown rump length as means of assessing maturity in first trimester of pregnancy. *Br Med J* 1973;**4**:28-31.
4. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. *Bull World Health Organ* 1987;**65**:663–737.
5. Gresham E, Byles JE, Bisquera A, Hure AJ. Effects of dietary interventions on neonatal and infant outcomes: a systematic review and meta-analysis. *Am J Clin Nutr* 2014;**100**:1298-1321.
6. Grieger JA, Clifton VL. A review of the impact of dietary intakes in human pregnancy on infant birthweight. *Nutrients* 2014;**7**:153-178.
7. Timmermans S, Jaddoe VW, Hofman A, Steegers-Theunissen RP, Steegers EA. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. *Br J Nutr* 2009;**102**:777-785.
8. M'hamdi HI, van Voorst SF, Pinxten W, Hilhorst MT, Steegers EA. Barriers in the Uptake and Delivery of Preconception Care: Exploring the Views of Care Providers. *Matern Child Health J* 2016. (in press)
9. Steegers-Theunissen RP, Steegers EA. Embryonic health: new insights, mHealth and personalised patient care. *Reprod Fertil Dev* 2015;**27**:712-715.
10. van Uitert EM, van der Elst-Otte N, Wilbers JJ, Exalto N, Willemsen SP, Eilers PH, Koning AH, Steegers EA, Steegers-Theunissen RP. Periconception maternal

characteristics and embryonic growth trajectories: the Rotterdam Predict study. *Hum Reprod* 2013;**28**:3188-3196.

11. Bottomley C, Daemen A, Mukri F, Papageorghiou AT, Kirk E, Pexsters A, De Moor B, Timmerman D, Bourne T. Assessing first trimester growth: the influence of ethnic background and maternal age. *Hum Reprod* 2009;**24**:284-290.
12. Mook-Kanamori DO, Steegers EA, Eilers PH, Raat H, Hofman A, Jaddoe VW. Risk factors and outcomes associated with first-trimester fetal growth restriction. *JAMA* 2010;**303**:527-534.
13. van Uitert EM, van Ginkel S, Willemsen SP, Lindemans J, Koning AH, Eilers PH, Exalto N, Laven JS, Steegers EA, Steegers-Theunissen RP. An optimal periconception maternal folate status for embryonic size: the Rotterdam Predict study. *BJOG* 2014;**121**:821-829.
14. Bouwland-Both MI, Steegers-Theunissen RP, Vujkovic M, Lesaffre EM, Mook-Kanamori DO, Hofman A, Lindemans J, Russcher H, Jaddoe VW, Steegers EA. A periconceptional energy-rich dietary pattern is associated with early fetal growth: the Generation R study. *BJOG* 2013;**120**:435-445.
15. Macklon NS, Geraedts JP, Fauser BC. Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. *Hum Reprod Update* 2002;**8**:333-343.
16. Steegers-Theunissen RP, Twigt J, Pestinger V, Sinclair KD. The periconceptional period, reproduction and long-term health of offspring: the importance of one-carbon metabolism. *Hum Reprod Update* 2013;**19**:640-655.
17. Verwoerd-Dikkeboom CM, Koning AH, Hop WC, Rousian M, Van Der Spek PJ, Exalto N, Steegers EA. Reliability of three-dimensional sonographic measurements in early pregnancy using virtual reality. *Ultrasound Obstet Gynecol* 2008;**32**:910-916.

18. Rousian M, Hop WC, Koning AH, van der Spek PJ, Exalto N, Steegers EA. First trimester brain ventricle fluid and embryonic volumes measured by three-dimensional ultrasound with the use of I-Space virtual reality. *Hum Reprod* 2013;**28**:1181-1189.
19. Hu FB, Rimm EB, Smith-Warner SA, Feskanich D, Stampfer MJ, Ascherio A, Sampson L, Willet WC. Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. *Am J Clin Nutr* 1999;**69**:243–249.
20. Steegers-Theunissen RP, Verheijden-Paulissen JJ, van Uitert EM, Wildhagen MF, Exalto N, Koning AH, Eggink AJ, Duvekot JJ, Laven JS, Tibboel D, Reiss I, Steegers EA. Cohort Profile: The Rotterdam Periconceptional Cohort (Predict Study). *Int J Epidemiol* 2016;**45**:374-381.
21. Rousian M, Koning AH, van Oppenraaij RH, Hop WC, Verwoerd-Dikkeboom CM, van der Spek PJ, Exalto N, Steegers EA. An innovative virtual reality technique for automated human embryonic volume measurements. *Hum Reprod* 2010;**25**:2210-2216.
22. Siebelink E, Geelen A, de Vries JH. Self-reported energy intake by FFQ compared with actual energy intake to maintain body weight in 516 adults. *Br J Nutr* 2011;**106**:274-281.
23. Verkleij-Hagoort AC, de Vries JH, Stegers MP, Lindemans J, Ursem NT, Steegers-Theunissen RP. Validation of the assessment of folate and vitamin B12 intake in women of reproductive age: the method of triads. *Eur J Clin Nutr* 2007;**61**:610-615.
24. Netherlands Nutrition Centre. NEVO-tabel; Nederlands Voedingsstoffenbestand 2011, RIVM/Voedingscentrum, Den Haag. 2011.
25. Vujkovic M, Ocke MC, van der Spek PJ, Yazdanpanah N, Steegers EA, Steegers-Theunissen RP. Maternal Western dietary patterns and the risk of developing a cleft lip with or without a cleft palate. *Obstet Gynecol* 2007;**110**:378-384.

26. Hoffmann K, Schulze MB, Achienkiewitz A, Nothlings U, Boeing H. Application of a new statistical method to derive dietary patterns in nutritional epidemiology. *Am J Epidemiol* 2004;**159**:935–944**.**

27. Ramirez-Zea M. Validation of three predictive equations for basal metabolic rate in adults. *Public Health Nutr* 2005;**8**:1213-1228.

28. Ioannou C, Sarris I, Salomon LJ, Papageorghiou AT. A review of fetal volumetry: the need for standardization and definitions in measurement methodology. *Ultrasound Obstet Gynecol* 2011;**38**:613-619.

29. Hammiche F, Vujkovic M, Wijburg W, de Vries JH, Macklon NS, Laven JS, Steegers-Theunissen RP. Increased preconception omega-3 polyunsaturated fatty acid intake improves embryo morphology. *Fertil Steril* 2011;**95**:1820-1823.

30. Drouillet P, Kaminski M, De Lauzon-Guillain B, Forhan A, Ducimetière P, Schweitzer M, Magnin G, Goua V, Thiébaugeorges O, Charles MA. Association between maternal seafood consumption before pregnancy and fetal growth: evidence for an association in overweight women. The EDEN mother-child cohort. *Paediatr Perinat Epidemiol* 2009;**23**:76-86.

31. Heppe DH, Steegers EA, Timmermans S, Breeijen Hd, Tiemeier H, Hofman A, Jaddoe VW. Maternal fish consumption, fetal growth and the risks of neonatal complications: the Generation R Study. *Br J Nutr* 2011;**105**:938-949.

32. Leventakou V, Roumeliotaki T, Martinez D, Barros H, Brantsaeter AL, Casas M, Charles MA, Cordier S, Eggesbø M, van Eijsden M, Forastiere F, Gehring U, Govarts E, Halldórsson TI, Hanke W, Haugen M, Heppe DH, Heude B, Inskip HM, Jaddoe VW, Jansen M, Kelleher C, Meltzer HM, Merletti F, Moltó-Puigmartí C, Mommers M, Murcia M, Oliveira A, Olsen SF, Pele F, Polanska K, Porta D, Richiardi L, Robinson SM, Stigum

H, Strøm M, Sunyer J, Thijs C, Viljoen K, Vrijkotte TG, Wijga AH, Kogevinas M, Vrijheid M, Chatzi L. Fish intake during pregnancy, fetal growth, and gestational length in 19 European birth cohort studies. *Am J Clin Nutr* 2014;**99**:506-516.

33. Cetin I, Alvino G, Cardellicchio M. Long chain fatty acids and dietary fats in fetal nutrition. *J Physiol* 2009;**587**:3441-3451.

34. Poudyal H, Panchal SK, Diwan V, Brown L. Omega-3 fatty acids and metabolic syndrome: effects and emerging mechanisms of action. *Prog Lipid Res* 2011;**50**:372–387.

35. Papadopoulou E, Caspersen IH, Kvalem HE, Knutsen HK, Duarte-Salles T, Alexander J, Meltzer HM, Kogevinas M, Brantsæter AL, Haugen M. Maternal dietary intake of dioxins and polychlorinated biphenyls and birth size in the Norwegian Mother and Child Cohort Study (MoBa). *Environ Int* 2013;**60**:209-216.

36. Xia W, Chiu YH, Williams PL, Gaskins AJ, Toth TL, Tanrikut C, Hauser R, Chavarro JE. Men's meat intake and treatment outcomes among couples undergoing assisted reproduction. *Fertil Steril* 2015;**104**:972-979.

37. Braga DP, Halpern G, Setti AS, Figueira RC, Iaconelli A Jr, Borges E Jr. The impact of food intake and social habits on embryo quality and the likelihood of blastocyst formation. *Reprod Biomed Online* 2015;**31**:30-38.

38. Knudsen VK, Orozova-Bekkevold IM, Mikkelsen TB, Wolff S, Olsen SF. Major dietary patterns in pregnancy and fetal growth. *Eur J Clin Nutr* 2008;**62**:463-470.

39. Shen Y, Wan H, Zhu J, Fang Z, Che L, Xu S, Lin Y, Li J, Wu D. Fish Oil and Olive Oil Supplementation in Late Pregnancy and Lactation Differentially Affect Oxidative Stress and Inflammation in Sows and Piglets. *Lipids* 2015;**50**:647-658.

40. Williams MA, Frederick IO, Qiu C, Meryman LJ, King IB, Walsh SW, Sorensen TK. Maternal erythrocyte omega-3 and omega-6 fatty acids, and plasma lipid concentrations, are associated with habitual dietary fish consumption in early pregnancy. *Clin Biochem* 2006;**39**:1063-1070.
41. Meher A, Randhir K, Mehendale S, Wagh G, Joshi S. Maternal Fatty Acids and Their Association with Birth Outcome: A Prospective Study. *PLoS One* 2016;**11**:e0147359 (in press).
42. Northstone K, Emmett PM, Rogers I. Dietary patterns in pregnancy and associations with nutrient intakes. *Br J Nutr* 2008;**99**:406-415.
43. Dumoulin JC, Land JA, Van Montfoort AP, Nelissen EC, Coonen E, Derhaag JG, Schreurs IL, Dunselman GA, Kester AD, Geraedts JP, Evers JL. Effect of in vitro culture of human embryos on birthweight of newborns. *Hum Reprod* 2010;**25**:605-612.
44. Nelissen EC, Van Montfoort AP, Smits LJ, Menheere PP, Evers JL, Coonen E, Derhaag JG, Peeters LL, Coumans AB, Dumoulin JC. IVF culture medium affects human intrauterine growth as early as the second trimester of pregnancy. *Hum Reprod* 2013;**28**:2067-2074.
45. Wale PL, Gardner DK. The effects of chemical and physical factors on mammalian embryo culture and their importance for the practice of assisted human reproduction. *Hum Reprod Update* 2016; **22**:2-22.
46. Baken L, van Heesch PN, Wildschut HI, Koning AH, van der Spek PJ, Steegers EA, Exalto N. First-trimester crown-rump length and embryonic volume of aneuploid fetuses measured in virtual reality. *Ultrasound Obstet Gynecol* 2013;**41**:521-525.

47. Hoffmann K, Schulze MB, Schienkiewitz A, Nöthlings U, Boeing H. Application of a new statistical method to derive dietary patterns in nutritional epidemiology. *Am J Epidemiol* 2004;**159**:935-944.
48. Crozier SR, Robinson SM, Godfrey KM, Cooper C, Inskip HM. Women's dietary patterns change little from before to during pregnancy. *J Nutr* 2009;**139**:1956-1963.
49. Beydoun MA, Kaufman JS, Ibrahim J, Satia JA, Heiss G. Measurement error adjustment in essential fatty acid intake from a food frequency questionnaire: alternative approaches and methods. *BMC Med Res Methodol* 2007;**7**:41.
50. Jaddoe VW, de Jonge LL, Hofman A, Franco OH, Steegers EA, Gaillard R. First trimester fetal growth restriction and cardiovascular risk factors in school age children: population based cohort study. *BMJ* 2014;**348**:g14.
51. van Uitert EM, Exalto N, Burton GJ, Willemsen SP, Koning AH, Eilers PH, Laven JS, Steegers EA, Steegers-Theunissen RP. Human embryonic growth trajectories and associations with fetal growth and birthweight. *Hum Reprod* 2013;**28**:1753-1761.

CONFLICT OF INTEREST

RST is CSO of the startup company Slimmere Zorg and CEO of eHealth Care Solutions.

TABLES

Table 1. Characteristics of the study subgroups and excluded pregnancies.

	Total study population (n=228)		**Excluded pregnancies (n=124)**		
MATERNAL CHARACTERISTICS		**M**		**M**	**p**
Age, y median (range)	32 (22-44)	0	30 (21-41) *	0	**0.01**
Geographical origin		1		5	0.05
Western, n(%)	205 (89.9)		105 (84.7)		
Non Western, n(%)	22 (9.6)		14 (11.3)		
Educational level		1		5	0.06
High, n(%)	134 (58.8)		66 (53.2)		
Intermediate, n(%)	89 (39.0)		49 (39.5)		
Low, n(%)	4 (1.8)		4 (3.2)		
BMI, kg/m^2 median (range)	24.2 (17.0-42.6)	1	25.8 * (17.8-45.0)	2	**0.02**
Nulliparous, n(%)	74 (32.5)	0	39 (32.5)	4	0.99
Alcohol use, n(%)	84 (37.0)	1	37 (31.9)	8	0.35
Periconceptional smoking, n(%)	33 (14.5)	1	20 (17.2)	8	0.51
Periconceptional folic acid/multivitamin use, n(%)	219 (97.3)	3	113 (94.2)	4	0.43
Chronic diseases, n(%)	26 (11.4)	0	21 (16.9)	0	**0**.15

Chronic diseases include cardiovascular, autoimmune, endocrinal and metabolic diseases. The comparison among groups was performed using Chi-square or exact tests for ordinal variables and Mann-Whitney U test for continuous variables.

M: missing values, BMI: body mass index.

Table 2. Relation between food groups and the identified dietary patterns expressed by factor loadings.

	High vegetables, fruits and grain dietary pattern	**High solid fat, snacks and sugars dietary pattern**	**High fish and olive oil, low meat dietary pattern**
Variance explained (%)	18.5	17.4	10.9
Cereals	0.141	-0.269	0.038
Olive Oil	0.231	0.205	0.469*
Solid fat	0.277	0.614*	-0.262
Fish	0.461*	-0.034	0.650*
Fruit	0.580*	-0.365*	-0.007
Grain	0.579*	0.379*	0.018
Vegetables	0.775*	-0.068	0.105
Meat	0.206	0.189	-0.750*
Snacks	-0.075	0.699*	0.029
Sugars	-0.079	0.620*	0.041

The factor loadings describe the food group contribution to each dietary pattern. The factor loadings with the highest absolute value are presented with an asterisk and were used for labelling (*).

Table 3. Effect estimates from the linear mixed model analysis for associations between maternal dietary patterns and embryonic crown-rump-length (CRL) and volume (EV) in the total study population and in strictly dated spontaneous and IVF/ICSI pregnancies.

DIETARY PATTERN	Effect estimates CRL β (95%CI), √mm		
	TOTAL STUDY POPULATION	**STRICTLY DATED SPONTANEOUS PREGNANCIES**	**IVF/ICSI PREGNANCIES**
High vegetables, fruits and grain			
Crude	+0.04 (-0.00, 0.08)	+0.05 (-0.02, 0.12)	+0.02 (-0.02, 0.06)
Fully adjusted	+0.04 (-0.01, 0.09)	+0.04 (-0.04, 0.12)	+0.03 (-0.02, 0.08)
High solid fat, snacks and sugars			
Crude	-0.02 (-0.07, 0.04)	-0.03 (-0.10, 0.05)	-0.00 (-0.06, 0.06)
Fully adjusted	-0.02 (-0.08, 0.04)	-0.03 (-0.11, 0.05)	-0.01 (-0.06, 0.05)
High fish and olive oil, low meat			
Crude	+0.03 (-0.01, 0.06)	+0.07 (0.01, 0.12) **	-0.03 (-0.07, 0.01)
Fully adjusted	+0.03 (-0.01, 0.07)	+0.07 (0.01, 0.13) **	-0.02 (-0.06, 0.02)
	Effect estimates EV β (95%CI), $\sqrt[3]{cm^3}$		
High vegetables, fruits and grain			
Crude	+0.01 (-0.01, 0.03)	+0.01 (-0.03, 0.05)	+0.01 (-0.01, 0.03)
Fully adjusted	+0.01 (-0.01, 0.03)	+0.01 (-0.03, 0.05)	+0.01 (-0.01, 0.03)
High solid fat, snacks, sugars			
Crude	-0.01 (-0.03, 0.01)	-0.02 (-0.06, 0.02)	-0.01 (-0.04, 0.03)
Fully adjusted	-0.02 (-0.04, 0.01)	-0.02 (-0.06, 0.02)	-0.01 (-0.04, 0.02)
High fish and olive oil, low meat			
Crude	+0.01 (-0.01, 0.03)	+0.03 (0.01, 0.05) *	-0.02 (-0.04, 0.00)
Fully adjusted	+0.01 (-0.01, 0.03)	+0.03 (0.01, 0.05) *	-0.02 (-0.04, 0.00)

Effect estimates represent the amount of change in square root CRL (√mm) and third root EV ($\sqrt[3]{cm^3}$) per unit of increase of factor score. Crude analysis is adjusted for gestational age and energy intake. Multivariable analysis is adjusted for all potential confounders (parity, alcohol use, smoking habit, folic acid/multivitamin supplement use, maternal age, BMI and comorbidity, fetal gender).

*$p<0.05$**;** ** $p<0.02$

CI: confidence interval.

LEGENDS FOR FIGURES

Figure 1. Flow chart of the study population. IUFD: intrauterine fetal death, FFQ: food frequency questionnaire, IUI: intrauterine insemination; IVF: *in vitro* fertilization, ICSI: intracytoplasmatic sperm injection; CRL: crown-rump length.

Figure 2. Fully adjusted linear mixed models for crown-rump length (CRL, n=614 measurements) (A) and embryonic volume (EV, n=554 measurements) (B) in relation to periconceptional maternal adherence to the 'high fish and olive oil, low meat' dietary pattern in the strictly dated spontaneous pregnancy subgroup. Maternal adherence to the 'high fish and olive oil, low meat' dietary pattern is expressed as +2 standard deviations (SD) (dashed line, strong adherence) and -2SD in factor scores (continuous line, weak adherence). Gestational age (GA) is expressed in days. Full adjustment for parity, alcohol use, smoking, folic acid/multivitamin supplement use, maternal age, BMI, comorbidity and fetal gender was performed.

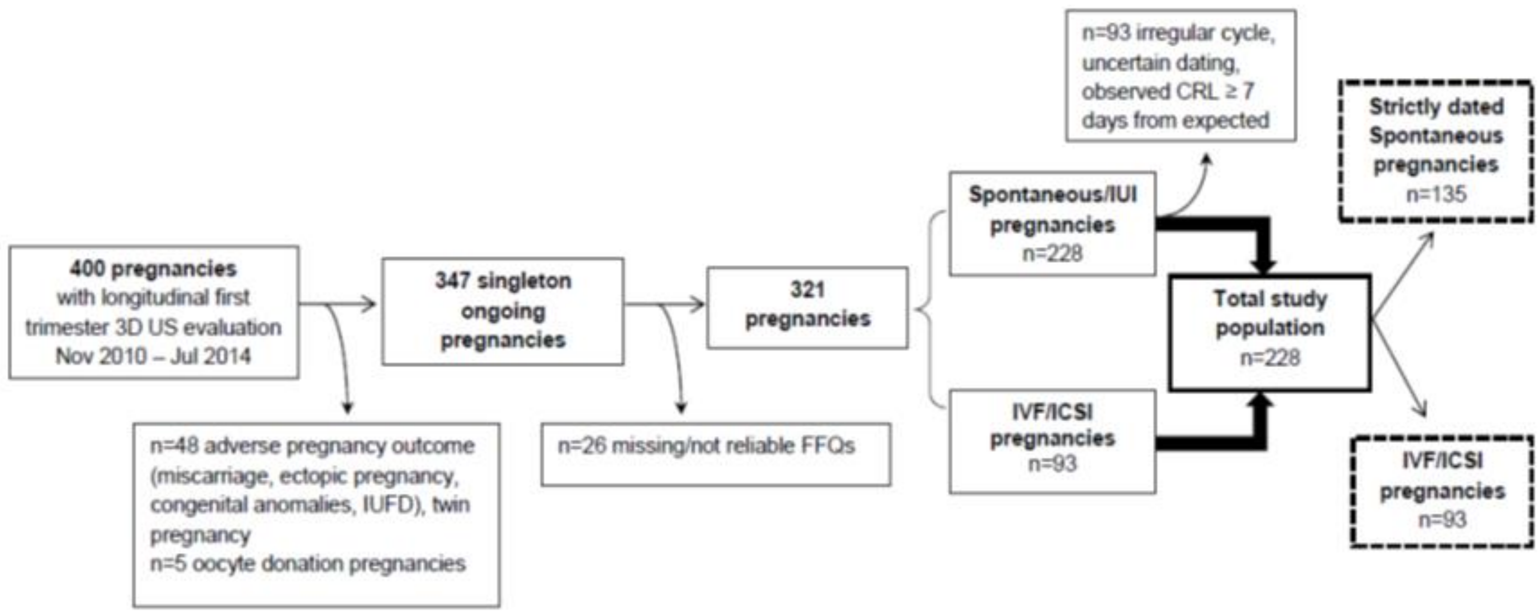
400 pregnancies
with longitudinal first
trimester 3D US evaluation
Nov 2010 – Jul 2014
n=48 adverse pregnancy outcome
(miscarriage, ectopic pregnancy,
congenital anomalies, IUFD), twin
pregnancy
n=5 oocyte donation pregnancies
347 singleton
ongoing
pregnancies
n=26 missing/not reliable FFQs
321
pregnancies
Spontaneous/IUI
pregnancies
n=228
n=93 irregular cycle,
uncertain dating,
observed CRL ≥ 7
days from expected
IVF/ICSI
pregnancies
n=93
Total study
population
n=228
Strictly dated
Spontaneous
pregnancies
n=135
IVF/ICSI
pregnancies
n=93

FIGURE 1

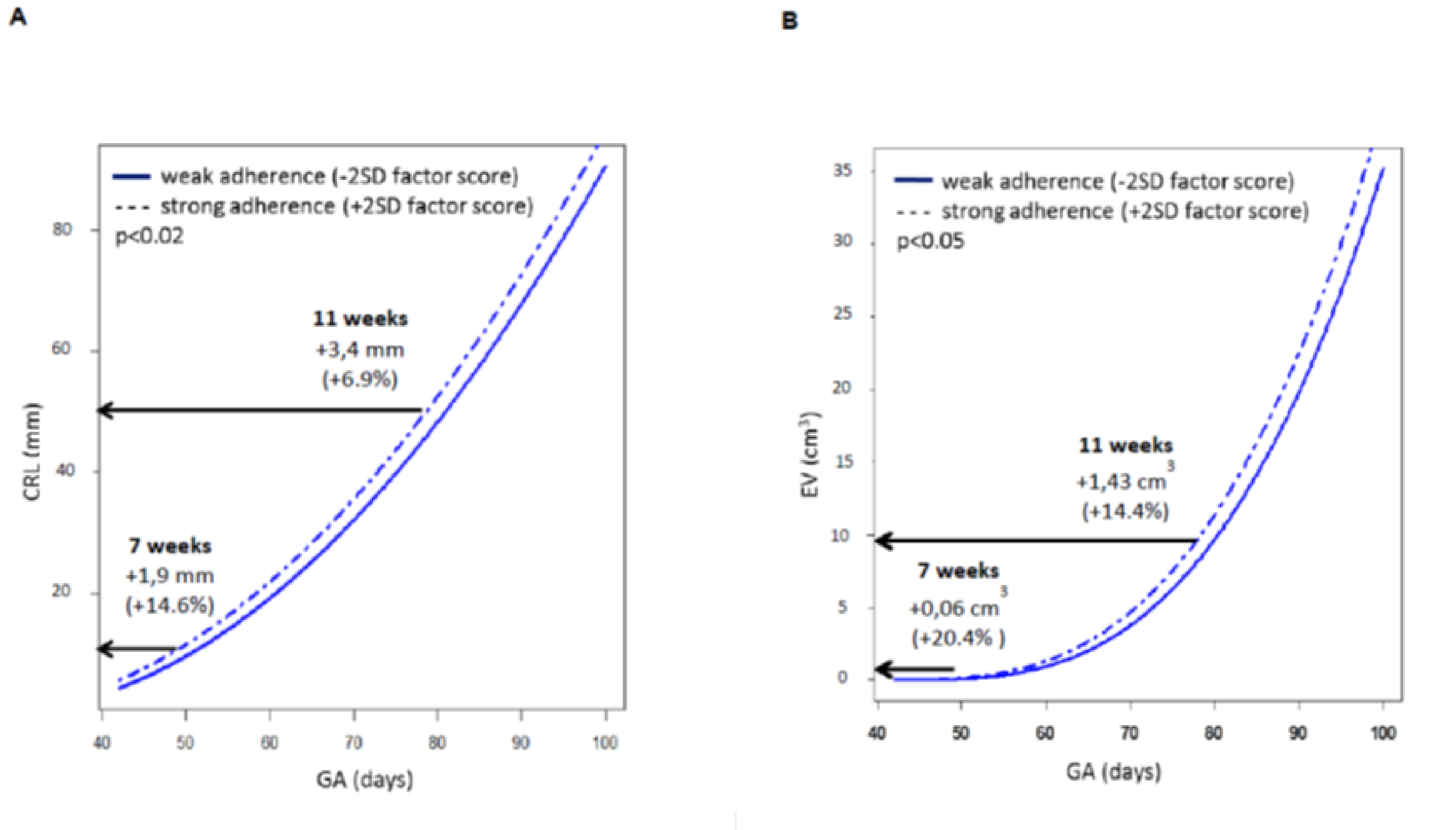
A
weak adherence (-2SD factor score)
strong adherence (+2SD factor score)
p<0.02
11 weeks
+3,4 mm
(+6.9%)
7 weeks
+1,9 mm
(+14.6%)
CRL (mm)
GA (days)
B
weak adherence (-2SD factor score)
strong adherence (+2SD factor score)
p<0.05
11 weeks
+1,43 cm3
(+14.4%)
7 weeks
+0,06 cm3
(+20.4%)
EV (cm3)
GA (days)

FIGURE 2