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Periconceptional maternal social, lifestyle and medical risk factors impair embryonic growth: The Rotterdam Periconceptional Cohort

BIOGRAPHY

Sofie van Zundert is a PhD candidate at the Departments of Obstetrics and Gynecology and Clinical Chemistry at the Erasmus MC, University Medical Center Rotterdam, the Netherlands. Her research focuses on the influence of periconceptional maternal social, lifestyle and medical risk factors (vulnerability markers) on biological processes and embryonic health.

Sofie K.M. van Zundert[1], Lenie van Rossem[1], Sten P. Willemsen[1,2], Lindsey van der Meer[1], Hiske E. Ernst-Smelt[1], Régine P.M. Steegers-Theunissen[1,*]

KEY MESSAGE

This study provides evidence that the accumulation of vulnerability markers impairs embryonic growth. This revelation emphasizes the importance of customized periconceptional behavioral change and support programs targeting vulnerable women as early as possible to optimize modifiable vulnerability markers and subsequent embryonic growth.

ABSTRACT

Research question: What is the association between the degree of a state of maternal vulnerability, determined by suboptimal periconceptional social, lifestyle and medical exposures and embryonic growth?

Design: In total, 555 pregnancies, comprising 324 naturally conceived pregnancies and 231 pregnancies conceived after IVF and intracytoplasmic sperm injection (ICSI) were included from the Rotterdam Periconceptional Cohort (Predict Study) between November 2010 and August 2018. Data on periconceptional social, lifestyle and medical exposures, i.e. vulnerability markers, were collected through self-administered questionnaires. To estimate embryonic growth, crown–rump length (CRL) and embryonic volume measurements were taken at 7, 9 and 11 weeks of gestation using three-dimensional ultrasound scans and virtual reality techniques.

Results: Exposure to two or more vulnerability markers was negatively associated with embryonic growth in naturally conceived pregnancies. The CRL and embryonic volume trajectories of embryos of women exposed to two vulnerability markers were reduced compared with those of women exposed to zero or one vulnerability marker (√CRL: β = –0.29 mm, 95% CI –0.56 to –0.02; $\sqrt[3]{}$EV: β = –0.14 cm^3, 95% CI –0.27 to –0.01). These associations were not found in pregnancies conceived after IVF or ICSI.

Conclusions: This study showed that a higher degree of the periconceptional state of maternal vulnerability is associated with reduced embryonic growth (CRL and embryonic volume) in naturally conceived pregnancies.

[1] Department of Obstetrics and Gynecology, Erasmus MC, University Medical Center, P O Box 2040, Rotterdam CA 3000, the Netherlands
[2] Department of Biostatistics, Erasmus MC, University Medical Center, P O Box 2040, Rotterdam CA 3000, the Netherlands

*Corresponding author: E-mail address: r.steegers@erasmusmc.nl (R. P. Steegers-Theunissen). https://doi.org/10.1016/j.rbmo.2022.02.011 1472-6483/
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KEYWORDS

Crown–rump length
Embryonic volume
Maternal vulnerability
Periconceptional period
Pregnancy

INTRODUCTION

In recent years, the importance of embryonic growth came to prominence after the finding that embryonic growth showed individual variation (*van Uitert et al., 2013a*). These differences affect fetal growth, birth outcomes, newborn health and health later in life (*Mook-Kanamori et al., 2010*; *Baken et al., 2013*; *van Uitert et al., 2013a*; *Jaddoe et al., 2014*). This evidence strongly adds to the theory of the Developmental Origin of Health and Disease (DOHaD) and emphasizes the effect of exposures in the periconceptional period on fetal development and future health of the offspring (*Hales and Barker, 2001*; *Gluckman et al., 2010*). Therefore, the periconceptional period is considered to be the most critical period in life during which the parental environment affects the developmental programming of offspring via mechanisms such as epigenetic modifications (*Steegers-Theunissen et al., 2013*).

Over the past few decades, various technological developments have contributed to accurate assessment of embryonic growth *in vivo*, which has opened new avenues for research (*Verwoerd-Dikkeboom et al., 2010*). Associations between maternal conditions, lifestyle exposures and embryonic growth have previously been studied (*Oostingh et al., 2019*). To date, studies have mainly focused on the influence of one single maternal risk factor at a time on embryonic growth, whereas vulnerable women are often exposed to multiple social, lifestyle and medical risk factors simultaneously.

The municipality of Rotterdam and the Department of Obstetrics and Gynecology of the Erasmus MC, University Medical Center Rotterdam, have been working together for many years to optimize care for vulnerable women of reproductive age. In 2019, they drafted a uniform definition of a state of maternal vulnerability (*van der Meer et al., 2020*). According to this definition, pregnant women are considered potentially vulnerable when they are exposed to one or more social, lifestyle or medical risk factor(s), defined as vulnerability marker(s).

Several studies have demonstrated that vulnerability markers predict the risk of adverse pregnancy outcomes (*Posthumus et al., 2016*; *Brembilla et al., 2019*). *Brembilla et al. (2019)* showed that vulnerability markers tend to accumulate and are subsequently associated with an increased risk of adverse pregnancy outcomes (*Brembilla et al., 2019*). Given that some of these vulnerability markers are modifiable and embryonic growth is important for newborn health and health later in life, it is particularly interesting to investigate the effect of accumulation of vulnerability markers on embryonic growth.

We hypothesize that a higher degree of the periconceptional state of maternal vulnerability impairs embryonic growth. Therefore, the aim of the present study was to investigate the association between the degree of the periconceptional state of maternal vulnerability, determined by the accumulation of vulnerability markers, and embryonic growth depicted by longitudinal crown–rump length (CRL) and embryonic volume measurements.

MATERIALS AND METHODS

Study population

This study was conducted using data from the Rotterdam Periconceptional Cohort (Predict Study), a longitudinal prospective periconceptional hospital-based cohort conducted at the Department of Obstetrics and Gynecology of the Erasmus MC, University Medical Center Rotterdam, the Netherlands. The follow-up continued until 1 year after delivery. A comprehensive description of the cohort has previously been published (*Steegers-Theunissen et al., 2016*; *Rousian et al., 2021*). Women aged 18 years or older, familiar with the Dutch language and planning pregnancy, or less than 10 weeks' pregnant (and their partners) were eligible for inclusion.

All procedures carried out in this study were conducted in accordance with the ethical standards of the local Medical Ethical Committee of the Erasmus MC, University Medical Center Rotterdam, the Central Committee on Research The Hague (MEC-2004-227; approved on 15 October 2004) (*Steegers-Theunissen et al., 2016*) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Furthermore, all participants gave written informed consent before inclusion.

Between November 2010 and August 2018, 2515 participants were recruited. The following were excluded from the analysis: pregnancies without three-dimensional ultrasound data (n = 1239), including multiple pregnancies (n = 64) and miscarriages (n = 125), terminated pregnancies (n = 17), pregnancies that ended in fetal or neonatal death (n = 21) or a congenital anomaly (n = 43), pregnancies conceived after oocyte donation (n = 18), naturally conceived pregnancies with an irregular menstrual cycle or an unknown first day of the last menstrual period (LMP) (n = 214) and pregnancies with missing data on vulnerability markers (n = 408). The resulting study population consisted of 555 pregnancies, comprising 324 naturally conceived pregnancies and 231 pregnancies conceived after IVF and intracytoplasmic sperm injection (IVF/ICSI) (FIGURE 1).

For naturally conceived pregnancies, gestational age was determined by the LMP and adjusted for length of the menstrual cycle for participants with a regular menstrual cycle shorter than 25 days or longer than 31 days. When gestational age determined by the LMP and via measurement of the CRL differed more than 7 days, gestational age was estimated by the CRL at the 9-week three-dimensional ultrasound scan. As CRL was one of the primary outcome measures of this study, these pregnancies were excluded to avoid bias. For pregnancies achieved through IVF/ICSI, gestational age was calculated by adding 14 days to the date of oocyte retrieval. If a cryopreserved embryo was used, gestational age was calculated by adding 19 days to the embryo transfer date, as thawed cryopreserved embryos were transferred at day 5 of embryo development.

Data collection

Participants completed a general questionnaire concerning periconceptional social, lifestyle and medical (risk) factors, and a standardized validated semi-quantitative food frequency questionnaire during the early first trimester (*Feunekes et al., 1993*). Completeness and consistency of these questionnaires were checked, and anthropometric measurements were obtained by trained researchers during the intake appointment at the hospital. Data on pregnancy outcomes were collected by means of a questionnaire

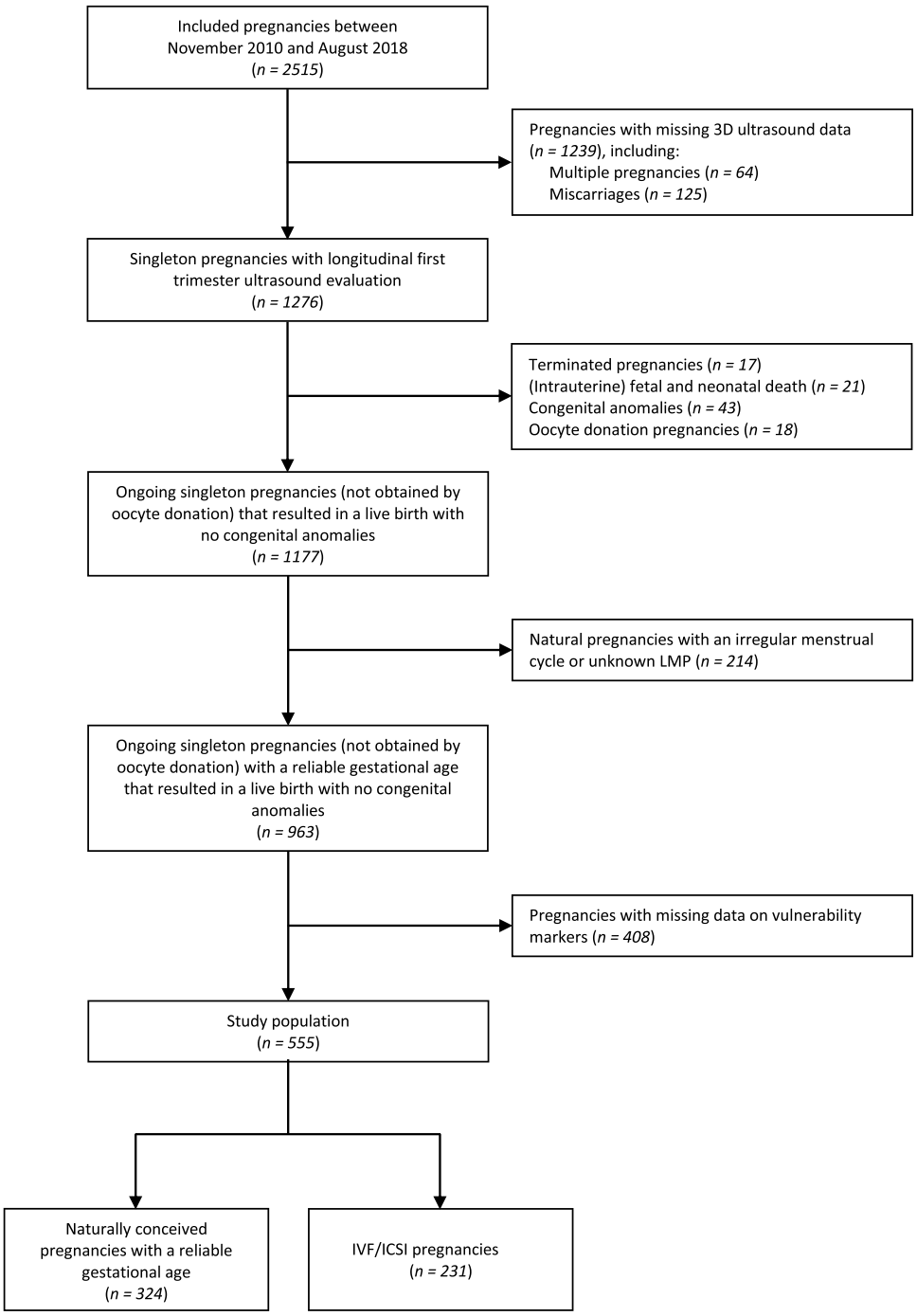

FIGURE 1 Study population. ICSI, intracytoplasmic sperm injection; LMP, last menstrual period; 3D, three-dimensional.

1 year after delivery and were verified through medical records.

Exposures

Periconceptional maternal social, lifestyle and medical risk factors were selected as markers for the periconceptional state of maternal vulnerability according to the recently published definition of a state of maternal vulnerability with some modifications (*van der Meer et al., 2020*). The 14 vulnerability markers that were available for this study were grouped into a social (n = 5) (extremely young or advanced age at conception, non-western background, single, deprived neighbourhood and low level of education), lifestyle (n = 5) (smoking, alcohol consumption, drug use, inadequate fruit and vegetable intake and inadequate physical activity) and medical domain (n = 4) (chronic diseases, any medication use, underweight or obesity and psychiatric disorders) (TABLE 1).

Maternal age at conception younger than 20 years and 40 years or older were considered extremely young and advanced, respectively (*Lean et al., 2017*). The background of women was categorized into western and non-western (*Alders, 2001*). A non-western background comprised African, Asian (except for

TABLE 1 VULNERABILITY MARKERS GROUPED INTO A SOCIAL, LIFESTYLE AND MEDICAL DOMAIN

Domains	Vulnerability markers
Social	Extremely young or advanced age at conception (<20 years or ≥40 years) Non-western background Single Deprived neighbourhood (NSS <–1) Low level of education (ISCED 0–2)
Lifestyle	Smoking Alcohol consumption Drug use Inadequate fruit and vegetable intake (<400 g/day) Inadequate physical activity (<150 MPW)
Medical	Chronic diseases Any medication use Underweight or obesity (BMI <18.5 kg/m^2 or BMI ≥30 kg/m^2) Psychiatric disorders

BMI, body mass index; ISCED, International Standard Classification of Education; MPW, minutes per week; NSS, Neighbourhood Status Score.

Indonesia and Japan), Latin-American, and Turkish background. On the basis of their socioeconomic position in Dutch society, women with an Indonesian or Japanese background were considered western. Women with an Indonesian background often have parents originating from the former Dutch East Indies, and most women and their families with a Japanese background are employees of Japanese companies. In addition, women from Europe (excluding Turkey), North America and Oceania were classified as western. The level of neighbourhood deprivation was based on the Neighbourhood Status Score as calculated by *The Netherlands Institute for Social Research (2017)*: a score less than –1 and a score greater than 1, corresponded to a deprived and non-deprived neighbourhood, respectively. Level of education was classified into three categories according to the International Standard Classification of Education (ISCED) (*United Nations Educational Scientific and Cultural Organization 2012*): low (ISCED 0–2), intermediate (ISCED 3–4) and high level of education (ISCED 5–8). A low level of education included early childhood education (ISCED 0), primary education (ISCED 1) and lower secondary education (ISCED 2).

Smoking, alcohol consumption and drug use included any consumption during the periconceptional period. Total fruit and vegetable intake less than 400 g per day was regarded as inadequate, as stated by the *World Health Organization (2002)*. Furthermore, inadequate physical activity was defined as less than 150 min of physical activity per week (*World Health Organization, 2010*).

Various medical conditions, all persistent or long lasting in their effects, fell under the umbrella of chronic diseases (*Bernell and Howard, 2016*). Any medication use included prescription and over-the-counter medication (*WHO Collaborating Centre for Drug Statistics Methodology 2020*). A Body Mass Index less than 18.5 kg/m^2 and 30 kg/m^2 or over were defined as underweight and obese, respectively (*World Health Organization, 2000*). Psychiatric disorders were classified according to the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-V) and verified using medical records (*American Psychiatric Association, 2013*).

Outcomes

Participants received three standardized three-dimensional ultrasound scans at 7, 9 and 11 weeks of gestation carried out by seven medical doctors, who were trained in carrying out first-trimester ultrasound scans, with a 6–12 MHz transvaginal probe using the GE Voluson E8 equipment and 4D View software (General Electric Medical Systems, Zipf, Austria). These three-dimensional ultrasound scans were stored as Cartesian volumes and visualized using the Barco I-Space virtual reality system at the Department of Bioinformatics of the Erasmus MC, University Medical Center Rotterdam, the Netherlands. The V-Scope software used in the Barco I-Space, allows efficient measurements of the embryo to be obtained by creating an interactive three-dimensional hologram of the ultrasound scan (*Koning et al., 2009*; *Rousian et al., 2010*). CRL measurements were repeated three times, and the average of these measurements was used. Embryonic volume measurements were carried out once using a semi-automatic method based on gray-scale differences. The accuracy and reliability of the embryonic volume measurements were studied previously and were proven to be outstanding (interobserver variability: intraclass correlation coefficient = 0.999, 95% CI 0.997 to 0.999; intraobserver variability: intraclass correlation coefficient = 0.999, 95% CI 0.998, 0.999) (*Rousian et al., 2010*).

Statistical analysis

On the basis of the distribution of the cumulative exposure to vulnerability markers (FIGURE 2), women were divided into five vulnerability categories with an ascending number of vulnerability marker exposures (zero or one, two, three, four and five or more). Baseline characteristics of the study population, stratified per vulnerability category, were studied. Continuous variables were presented as median with an interquartile range (IQR) and categorical variables as number of individuals and percentages. To determine which vulnerability markers were overrepresented in the highest vulnerability category, the phi coefficient was calculated as a measure of the association between exposure to a single vulnerability marker and exposure to five or more other vulnerability markers.

Linear mixed models were used to determine the association between the degree of the periconceptional state of maternal vulnerability and embryonic growth. Square root transformations of CRL and cube root transformations of embryonic volume were carried out, leading to distributions that were approximately normal given the covariates and a linear association with gestational age.

In the crude model (model 1), the association between the degree of the periconceptional state of maternal vulnerability and embryonic growth was adjusted for gestational age. Model 2 was additionally adjusted for fetal sex, parity and folic acid supplement use based on previous research (*Bukowski et al., 2007*; *Shah, 2010*; *van Uitert et al., 2013b*; *Van Dijk et al., 2018*). A subgroup analysis was carried out in naturally conceived pregnancies and IVF/ICSI pregnancies, to account for differences in the accuracy of pregnancy dating and occurrence of risk factors (*Qin et al., 2016*).

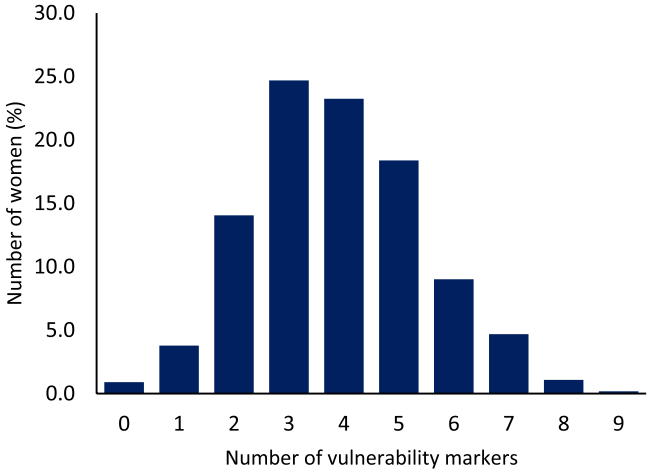

FIGURE 2 Distribution of the cumulative exposure to vulnerability markers among the total study population.

IBM SPSS Statistics for Windows, Version 25 (IBM Corporation, Armonk, NY, USA), was used for data analysis. $P \leq 0.05$ was considered to be statistically significant, and reported results from linear mixed models as effect estimates with 95% confidence intervals.

RESULTS

Maternal baseline characteristics

Baseline characteristics of the total study population, including stratification by vulnerability category, are presented in TABLE 2. The median age at conception was 32.3 years (IQR 29.4–35.4) with no maternal age at conception below 20 years. Most of the women had a western background (n = 487 [87.7%]), had a partner (n = 546 [98.4%]), lived in a non-deprived neighbourhood (n = 448 [80.7%]) and were highly educated (n = 358 [64.5%]). A total of 78 (14.1%) women smoked, 179 (32.3%) consumed alcohol and six (1.1%) used drugs. Fruit and vegetable intake (n = 381 [68.6%]) and physical activity (n = 431 [77.7%]) were predominantly inadequate. More than one-half of the study population suffered from at least one chronic disease (n = 339 [61.1%]) and used medication (n = 342 [61.6%]). The median Body Mass Index was 24.3 kg/m^2 (IQR 21.9–28.1 kg/m^2) and 11 (2.0%) women were underweight, whereas 96 (17.3%) were obese. A minority of the women were diagnosed with a psychiatric disorder (n = 55 [9.9%]).

As can be concluded from TABLE 2, the prevalence of most single vulnerability markers increased in proportion to the number of vulnerability marker exposures. If women were exposed to only one vulnerability marker, this was most often inadequate fruit and vegetable intake or inadequate physical activity. The vulnerability markers deprived neighbourhood (φ = 0.11, P = 0.010), low level of education (φ = 0.12, P = 0.005), smoking (φ = 0.16, $P < 0.001$), drug use (φ = 0.11, P = 0.009), obesity (φ = 0.13, P = 0.002) and psychiatric disorders (φ = 0.11, P = 0.012) were overrepresented in women exposed to five or more other vulnerability markers. Furthermore, in this group, women who conceived naturally prevailed (70.8%). Conversely, women exposed to zero or one vulnerability marker predominantly conceived after IVF or ICSI (57.7%).

The baseline characteristics of the excluded population are shown in Supplementary Table 1. The excluded population has slightly less favourable baseline characteristics and consists of more naturally conceived pregnancies compared with the study population.

Distribution of vulnerability markers

As presented in FIGURE 2, the cumulative exposure to vulnerability markers among the study population shows a near normal distribution. Five women (0.9%) were not exposed to any vulnerability marker, whereas one woman (0.2%) was exposed to nine of the 14 vulnerability markers. Most women were exposed to two or more vulnerability markers (n = 451 [81.3%]).

Embryonic growth

The effect estimates from the linear mixed models of the associations between each vulnerability category and embryonic growth in the total study population, naturally conceived pregnancies and IVF/ICSI pregnancies are presented in TABLE 3. The corresponding embryonic growth curves are presented in FIGURE 3. In the section below, a detailed description is given of these results in the total study population and stratified by mode of conception.

As the crude effect estimates (model 1) and the adjusted effect estimates (model 2) concerning the CRL and embryonic volume trajectories were comparable, only the results of model 2 are described. The median CRL and embryonic volume were 11.88 mm and 0.20 cm^3 at 7 weeks of gestation, and 49.41 mm and 10.41 cm^3 at 11 weeks of gestation.

Total study population

In general, model 2 showed a consistent negative, but non-significant, association between the degree of the periconceptional state of maternal vulnerability and embryonic growth in the total study population (TABLE 3). More specifically, exposure to two or more vulnerability markers was associated with reduced CRL and embryonic volume trajectories compared with exposure to zero or one vulnerability marker. The effect estimates for both CRL and embryonic volume trajectories differed marginally between the vulnerability categories (TABLE 3) (√CRL two vulnerability markers: β = –0.13 mm, 95% CI –0.28 to 0.01; √CRL five or more vulnerability markers: β = –0.11 mm, 95% CI –0.24 to 0.02; $\sqrt[3]{}$EV two vulnerability markers: β = –0.06 cm^3, 95% CI –0.13 to 0.01; $\sqrt[3]{}$EV five or more vulnerability markers = –0.04 cm^3, 95% CI –0.11 to 0.03. As an illustration, at a gestational age of 11 weeks, the CRL and embryonic volume of embryos of women with two vulnerability marker exposures were 1.79 mm (3.9%) and 0.24 cm^3 (5.6%) smaller, and the CRL and embryonic volume of embryos of women with five or more vulnerability marker exposures were 1.44 mm (3.2%) and 0.16 cm^3 (3.8%) smaller than those of women exposed to zero or one vulnerability marker.

Naturally conceived pregnancies

The negative association between the degree of the periconceptional state of maternal vulnerability and embryonic growth was most pronounced in naturally conceived pregnancies. Again, the effect estimates of the different vulnerability categories were comparable

TABLE 2 BASELINE CHARACTERISTICS OF THE STUDY POPULATION, STRATIFIED BY VULNERABILITY CATEGORY

Domain	Baseline characteristics (vulnerability markers and other variables)	Study population (*n = 555*)	Zero or one vulnerability marker (*n = 26*)	Two vulnerability markers (*n = 78*)	Three vulnerability markers (*n = 137*)	Four vulnerability markers (*n = 129*)	Five or more vulnerability markers (*n = 185*)
Social	Age at conception, years,	32.3 (29.4–35.4)	32.9 (30.1–35.5)	32.2 (29.3–34.7)	33.1 (30.0–35.9)	32.4 (30.0–35.8)	31.5 (24.7–38.3)
	≥40	25 (4.5)	0 (0)	2 (2.6)	5 (3.6)	9 (7.0)	9 (4.9)
	20–39	530 (95.5)	26 (100)	76 (97.4)	132 (96.4)	120 (93)	176 (95.1)
	Background,						
	Non-western	68 (12.3)	0 (0)	2 (2.6)	7 (5.1)	13 (10.1)	46 (24.9)
	Western	487 (87.7)	26 (100)	76 (97.4)	130 (94.9)	116 (89.9)	139 (75.1)
	Relationship,						
	Single	9 (1.6)	0 (0)	0 (0)	1 (0.7)	2 (1.6)	6 (3.2)
	Partner	546 (98.4)	26 (100)	78 (100)	136 (99.3)	127 (98.4)	179 (96.8)
	Neighbourhood (NSS)	0.10 (–0.94 to 0.72)	0.03 (–0.70 to 1.09)	0.49 (–0.11 to 1.03)	0.43 (–0.44 to1.03)	0.01 (–0.78 to 0.60)	–0.64 (–1.67 to 0.57)
	Deprived (< –1)	107 (19.3)	2 (7.7)	1 (1.3)	17 (12.4)	18 (14.0)	69 (37.3)
	Non-deprived (≥ –1)	448 (80.7)	24 (92.3)	77 (98.7)	120 (87.6)	111 (86.0)	116 (62.7)
	Level of education (ISCED)						
	Low (0–2)	26 (4.7)	0 (0)	2 (2.6)	2 (1.5)	2 (1.6)	20 (10.8)
	Intermediate (3–4)	171 (30.8)	6 (23.1)	22 (28.2)	43 (31.4)	39 (30.2)	61 (33.0)
	High (5–8)	358 (64.5)	20 (76.9)	54 (69.2)	92 (67.2)	88 (68.2)	104 (56.2)
Lifestyle	Smoking						
	Yes	78 (14.1)	0 (0)	0 (0)	5 (3.6)	13 (10.1)	60 (32.4)
	No	477 (85.9)	26 (100)	78 (100)	132 (96.4)	116 (89.9)	125 (67.6)
	Alcohol consumption						
	Yes	179 (32.3)	1 (3.8)	8 (10.3)	33 (24.1)	44 (34.1)	93 (50.3)
	No	376 (67.7)	25 (96.2)	70 (89.7)	104 (75.9)	85 (65.9)	92 (49.7)
	Drug use						
	Yes	6 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	6 (3.2)
	No	549 (98.9)	26 (100)	78 (100)	137 (100)	129 (100)	179 (96.8)
	Fruit and vegetable intake (g/day)	328.1 (220.7–431.9)	488.8 (411.3–594.9)	411.2 (282.5–509.0)	358.0 (279.7–448.0)	323.9 (211.0–396.7)	265.4 (160.1–358.4)
	Inadequate (<400)	381 (68.6)	5 (19.2)	36 (46.2)	82 (59.9)	98 (76.0)	160 (86.5)
	Adequate (≥400)	174 (31.4)	21 (80.8)	42 (53.8)	55 (40.1)	31 (24.0)	25 (13.5)
	Physical activity (MPW)						
	Inadequate (<150)	431 (77.7)	7 (26.9)	43 (55.1)	102 (74.5)	110 (85.3)	169 (91.4)
	Adequate (≥150)	124 (22.3)	19 (73.1)	35 (44.9)	35 (25.5)	19 (14.7)	16 (8.6)
	Folic acid supplement use[a]						
	Inadequate[b]	90 (16.2)	1 (3.8)	0 (0)	17 (12.4)	20 (15.5)	52 (28.1)
	Adequate	464 (83.6)	25 (96.2)	78 (100)	120 (87.6)	109 (84.5)	132 (71.4)
Medical	Chronic diseases						
	Multiple	125 (22.5)	1 (3.8)	7 (9.0)	28 (20.4)	34 (26.4)	55 (29.7)
	One	214 (38.6)	2 (7.7)	18 (23.1)	44 (32.1)	55 (42.6)	95 (51.4)
	None	216 (38.9)	23 (88.5)	53 (67.9)	65 (47.4)	40 (31.0)	35 (18.9)
	Any medication use						
	Multiple	169 (30.5)	1 (3.8)	13 (16.7)	33 (24.1)	45 (34.9)	77 (41.6)
	One	173 (31.2)	1 (3.8)	19 (24.4)	35 (25.5)	45 (34.9)	73 (39.5)
	None	213 (38.4)	24 (92.3)	46 (59.0)	69 (50.4)	39 (30.2)	35 (18.9)
	BMI, kg/m^2	24.3 (21.9–28.1)	22.4 (20.7–24.5)	23.1 (21.5–25.7)	24.1 (22.1–26.7)	23.5 (21.7–27.5)	26.8 (22.7–30.9)
	<18.5	11 (2.0)	0 (0)	0 (0)	3 (2.2)	0 (0)	8 (4.3)
	18.5–30	448 (80.7)	26 (100)	74 (94.9)	123 (89.8)	111 (86.0)	114 (61.6)
	≥30	96 (17.3)	0 (0)	4 (5.1)	11 (8.0)	18 (14.0)	63 (34.1)
	Psychiatric disorders						
	Multiple	14 (2.5)	1 (3.8)	1 (1.3)	0 (0)	1 (0.8)	11 (5.9)
	One	41 (7.4)	0 (0)	0 (0)	3 (2.2)	9 (7.0)	29 (15.7)
	None	500 (90.1)	25 (96.2)	77 (98.7)	134 (97.8)	119 (92.2)	145 (78.4)
	Parity						
	Primiparous	290 (52.3)	15 (57.7)	44 (56.4)	64 (46.7)	75 (58.1)	92 (49.7)
	1–2	247 (44.5)	10 (38.5)	34 (43.6)	71 (51.8)	50 (38.8)	82 (44.3)
	≥3	18 (3.2)	1 (3.8)	0 (0)	2 (1.5)	4 (3.1)	11 (5.9)
	Mode of conception						
	IVF/ICSI	231 (41.6)	15 (57.7)	43 (55.1)	63 (46.0)	56 (43.4)	54 (29.2)
	Natural	324 (58.4)	11 (42.3)	35 (44.9)	74 (54.0)	73 (56.6)	131 (70.8)

Continuous variables are presented as median with an interquartile range (IQR) and categorical variables as number of individuals and percentages.

[a]Data on folic acid supplement use are not available for one participant.

[b]No or post-conception initiation of folic acid supplement use.

BMI, Body Mass Index; ICSI, intracytoplasmic sperm injection; ISCED, International Standard Classification of Education; MPW, minutes per week; NSS, Neighbourhood Status Score.

TABLE 3 EFFECT ESTIMATES FROM LINEAR MIXED MODELS OF THE ASSOCIATIONS BETWEEN EACH VULNERABILITY CATEGORY AND EMBRYONIC GROWTH IN THE TOTAL STUDY POPULATION, NATURALLY CONCEIVED PREGNANCIES AND IVF AND ICSI PREGNANCIES

Vulnerability category	β (95% CI)		
	Total study population	**Natural pregnancies**	**IVF/ICSI pregnancies**
√CRL, mm	**(*n* = 555)**	**(*n* = 324)**	**(*n* = 231)**
Zero or one vulnerability marker			
Model 1	0.00 (reference)	0.00 (reference)	0.00 (reference)
Model 2	0.00 (reference)	0.00 (reference)	0.00 (reference)
Two vulnerability markers			
Model 1	−0.13 (−0.27 to 0.02)	−0.27 (−0.54 to 0.00)	−0.03 (−0.14 to 0.07)
Model 2	−0.13 (−0.28 to 0.01)	−0.29[a] (−0.56 to −0.02)	−0.03 (−0.14 to 0.07)
Three vulnerability markers			
Model 1	−0.14[b] (−0.28 to −0.01)	−0.30[c] (−0.55 to −0.04)	−0.02 (−0.12 to 0.08)
Model 2	−0.13 (−0.26 to 0.00)	−0.29[d] (−0.55 to −0.04)	−0.01 (−0.11 to 0.09)
Four vulnerability markers			
Model 1	−0.12 (−0.25 to 0.02)	−0.27[e] (−0.53 to −0.02)	0.01 (−0.09 to 0.11)
Model 2	−0.10 (−0.23 to 0.03)	−0.25 (−0.50 to 0.00)	0.00 (−0.10 to 0.10)
Five or more vulnerability markers			
Model 1	−0.15[f] (−0.28 to −0.01)	−0.28[g] (−0.52 to −0.03)	−0.03 (−0.13 to 0.08)
Model 2	−0.11 (−0.24 to 0.02)	−0.23 (−0.47 to 0.02)	−0.04 (−0.14 to 0.07)
$\sqrt[3]{EV}$, cm^{3}[h]	(*n* = 545)	(*n* = 319)	(*n* = 226)
Zero or one vulnerability marker			
Model 1	0.00 (reference)	0.00 (reference)	0.00 (reference)
Model 2	0.00 (reference)	0.00 (reference)	0.00 (reference)
Two vulnerability markers			
Model 1	−0.06 (−0.13 to 0.02)	−0.13 (−0.26 to 0.00)	−0.01 (−0.08 to 0.06)
Model 2	−0.06 (−0.13 to 0.01)	−0.14[i] (−0.27 to −0.01)	−0.01 (−0.07 to 0.06)
Three vulnerability markers			
Model 1	−0.05 (−0.12 to 0.02)	−0.12 (−0.24 to 0.01)	0.01 (−0.06 to 0.07)
Model 2	−0.04 (−0.11 to 0.02)	−0.11 (−0.23 to 0.01)	0.01 (−0.05 to 0.07)
Four vulnerability markers			
Model 1	−0.06 (−0.12 to 0.01)	−0.13[j] (−0.25 to −0.01)	0.01 (−0.06 to 0.08)
Model 2	−0.05 (−0.12 to 0.02)	−0.12[k] (−0.24 to −0.00)	0.00 (−0.06 to 0.07)
Five or more vulnerability markers			
Model 1	−0.06 (−0.12 to 0.01)	−0.11 (−0.23 to 0.00)	0.00 (−0.07 to 0.07)
Model 2	−0.04 (−0.11 to 0.03)	−0.09 (−0.21 to 0.03)	−0.01 (−0.07 to 0.06)

Model 1 is adjusted for gestational age.

Model 2 is additionally adjusted for fetal sex, parity and folic acid supplement use.

[a]$P = 0.035$.

[b]$P = 0.040$.

[c]$P = 0.022$.

[d]$P = 0.023$.

[e]$P = 0.037$.

[f]$P = 0.031$.

[g]$P = 0.029$.

[h]Data on embryonic volume measurements are not available for 10 participants.

[i]$P = 0.038$.

[j]$P = 0.037$.

[k]$P = 0.049$.

CRL, crown–rump length; EV, embryonic volume; ICSI, intracytoplasmic sperm injection.

(TABLE 3): √CRL two vulnerability markers: β = −0.29 mm (95% CI −0.56 to −0.02); √CRL five or more vulnerability markers: β = −0.23 mm (95% CI −0.47 to 0.02); $\sqrt[3]{EV}$ two vulnerability markers: β = −0.14 cm^3 (95% CI −0.27 to −0.01); $\sqrt[3]{EV}$ five or more vulnerability markers: β = −0.09 cm^3 (95% CI −0.21 to 0.03). In other words, these associations mean that the CRL and embryonic volume of embryos of women with five or more vulnerability marker exposures was 1.44 mm (13.4%) and 0.06 cm^3 (45.8%) smaller at 7 weeks of gestation, and 3.07 mm (6.5%) and 1.16 cm^3 (12.6%) smaller at 11 weeks of gestation compared with those women exposed to zero or one vulnerability marker (FIGURE 3A and FIGURE 3B).

IVF/ICSI pregnancies

In pregnancies conceived after IVF or ICSI, the effect estimates of the associations between the degree of the periconceptional state of maternal

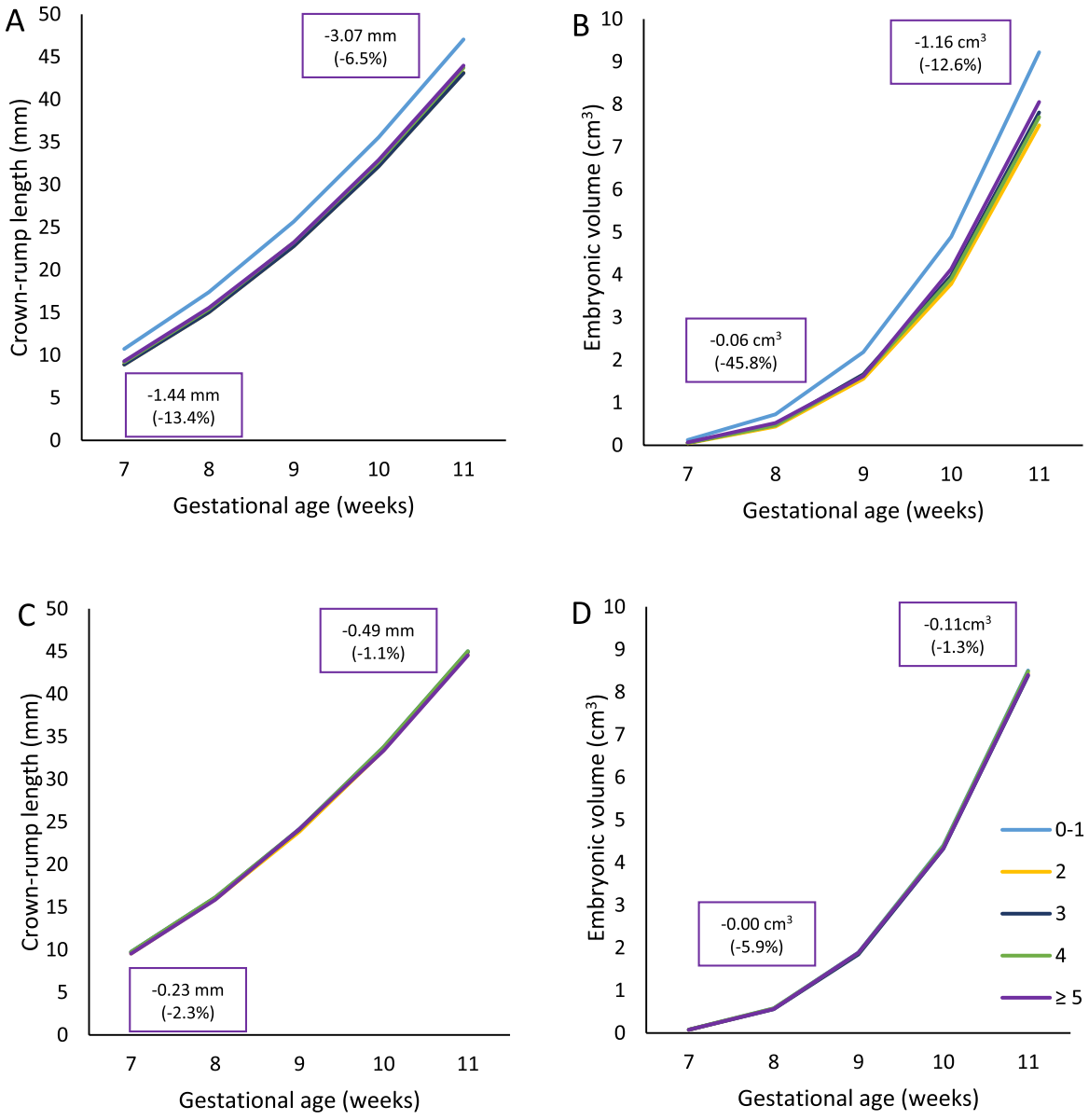

FIGURE 3 Embryonic growth (adjusted for gestational age, fetal sex, parity and folic acid supplement use) based on crown–rump length (CRL) and embryonic volume measurements in naturally conceived pregnancies (A and B) and IVF and ICSI pregnancies (C and D) for each vulnerability category. The difference in CRL and embryonic volume between embryos of women exposed to five or more vulnerability markers and women exposed to zero or one vulnerability marker is shown in mm and cm^3 and percentages, at 7 and 11 weeks of gestation, respectively.

vulnerability and the CRL and embryonic volume trajectories were close to zero and not statistically significant (TABLE 3, FIGURE 3C and FIGURE 3D).

DISCUSSION

Main findings

Our findings suggest that a higher degree of the periconceptional state of maternal vulnerability, in particular exposure to two or more vulnerability markers, is associated with reduced embryonic growth in naturally conceived pregnancies. We were not able to find an association between the degree of the periconceptional maternal state of vulnerability and embryonic growth in IVF/ICSI pregnancies, possibly because of a 'healthy' cohort and limited power.

Strengths and limitations

The major strength of this study is its longitudinal prospective periconceptional hospital-based design, which allowed for multiple CRL and embryonic volume measurements within one pregnancy and inclusion of a large number of participants. Additionally, the longitudinal CRL and embryonic volume measurements were obtained by experienced researchers using three-dimensional ultrasound scans and virtual-reality techniques, which provided accurate and reliable measurements of embryonic growth (*Rousian et al., 2010*). Furthermore, 14 different periconceptional maternal social, lifestyle and medical risk factors to determine the periconceptional state of maternal vulnerability were included (*van der Meer et al., 2020*). Other strengths are

the use of the standardized validated food frequency questionnaire and the Neighbourhood Status Score, and the verification of psychiatric disorders using medical records.

Some limitations of the present study also need to be acknowledged. First, the vulnerability categories were based on the number of vulnerability markers women were exposed to, without weighing them individually. Although a weighted scoring model may be more accurate, our approach is pragmatic, and the results can be easily translated into clinical practice. Second, social protective factors were not included in this study, which may mitigate the association between the degree of the periconceptional state of maternal vulnerability and embryonic growth. The inclusion of these social protective factors would have led to more precise estimates. Unfortunately, data on these factors were not available. Third, using the LMP to determine the gestational age of naturally conceived pregnancies (in the presence of a regular menstrual cycle) could have introduced confounding bias. To reduce the effects of variations in the timing of ovulation, however, we have adjusted the gestational age for the length of the menstrual cycle when the menstrual cycle was regular but differed more than 3 days from 28 days. Fourth, adjustment for multiple testing was not applied because of the exploratory character of the study. Finally, the generalizability of our results may be limited, as women were included in a tertiary care center. Therefore, our study should be repeated in a population-based periconceptional cohort to determine the external validity of our findings.

Interpretation

Initial research in this field has mainly focused on associations between single vulnerability markers and embryonic growth rather than on the association between the accumulation of vulnerability markers and embryonic growth. In fact, the effect estimates of the association between exposure to two vulnerability markers and the CRL trajectory (√CRL: β = –0.29 mm, 95% CI –0.56 to –0.02) is comparable to that of smoking 10 or more cigarettes a day and the CRL trajectory (√CRL: β = –0.21 mm, 95% CI –0.42 to –0.01) (*van Uitert et al., 2013b*). Compared with the effect estimates of the associations between single vulnerability markers and the embryonic volume trajectory, the effect estimates of the associations with the accumulation of vulnerability markers are larger. For instance, the $\sqrt[3]{}$EV of embryos of women who consume alcohol periconceptionally is reduced by 0.01 cm^3 (95% CI –0.04 to 0.03) compared with that of non-alcohol consumers. Compared with women who are exposed to zero or one vulnerability marker, the $\sqrt[3]{}$EV of embryos of women who are exposed to two vulnerability markers is reduced by 0.14 cm^3 (95% CI –0.27 to –0.01) (*Van Dijk et al., 2018*).

The heterogeneity of vulnerability makers complicates elucidating the exact mechanism by which the degree of the periconceptional state of maternal vulnerability affects embryonic growth. A potential mechanism underlying this association is derangement of epigenetic reprogramming during the periconceptional period caused by excessive cell multiplication and, therefore, the need for essential nutrients (*Nafee et al., 2008*). Exposure to vulnerability markers affects many pathways, including one-carbon metabolism providing one-carbon moieties for epigenetic modifications, such as DNA methylation. A reduced availability of these moieties, derived from nutrition, can derange the programming and subsequent expression of genes involved in embryonic growth and development (*Steegers-Theunissen et al., 2013*). Maternal smoking is an illustration of a widely studied vulnerability marker for DNA methylation. Previous research has demonstrated that maternal smoking directly affects the proteins for the establishment of the methyl moieties to the DNA (*Fragou et al., 2019*). Sustained maternal smoking after the first trimester is inversely associated with methylation of the insulin growth factor 2 differentially methylated region (IGF2DMR), which plays an important role in fetal growth (*Bouwland-Both et al., 2015*).

In the present study, no dose-effect relationship between the number of vulnerability markers women were exposed to and reduction of embryonic growth is demonstrated. This can be explained by the inverse associations between different vulnerability markers and embryonic growth that counterbalance each other. As an illustration, cardiovascular risk profiles, smoking 10 or more cigarettes a day and consuming alcohol during the periconceptional period are associated with reduced embryonic growth, whereas a higher maternal age and a black ethnic origin are associated with increased embryonic growth (*Bottomley et al., 2009*; *Mook-Kanamori et al., 2010*; *van Uitert et al., 2013b*; *Van Dijk et al., 2018*).

Remarkably, this study shows no association between the degree of the periconceptional state of maternal vulnerability and embryonic growth in pregnancies conceived after assisted reproductive technology. Our explanation is that subfertility itself and assisted reproductive technology overrule the association between the degree of the periconceptional state of maternal vulnerability and embryonic growth. Another explanation is the different distribution of vulnerability markers in these women.

Previous studies have shown that suboptimal embryonic growth is associated with reduced fetal growth, adverse birth outcomes and an increased risk of non-communicable diseases in offspring (*Mook-Kanamori et al., 2010*; *Baken et al., 2013*; *van Uitert et al., 2013a*; *Jaddoe et al., 2014*). This evidence highlights the importance of identifying vulnerable women as early as possible to reduce periconceptional exposure to vulnerability markers through targeted interventions.

In conclusion, the results of this study provide evidence that a higher degree of the periconceptional state of maternal vulnerability is negatively associated with embryonic growth in naturally conceived pregnancies. Exposure to two or more vulnerability markers is associated with reduced CRL and embryonic volume trajectories. The absence of these associations in IVF/ICSI pregnancies might be due to a 'healthy' cohort and limited power. More research should be undertaken to elucidate the mechanisms underlying these associations, and to develop targeted (lifestyle) interventions.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2022.02.011.

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