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# BMJ Open

## The impact of Bariatric surgery on EmbrYONic, foetal and placental Development (BEYOND): A prospective cohort study embedded in the Rotterdam periconception cohort

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3 1 **The impact of Bariatric surgery on EmbrYONic, foetal and placental Development (BEYOND): A**  
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5 2 **prospective cohort study embedded in the Rotterdam periconception cohort**  
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3 16 **Abstract**  
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5 17 **Introduction:** The worldwide obesity epidemic has resulted in a rise of bariatric surgery in women of  
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7 18 reproductive age which can lead to 'iatrogenic undernutrition'. Long lasting undernutrition can affect  
8  
9 19 maternal health, pregnancy outcomes and offspring. We hypothesise that embryonic and placental  
10  
11 20 growth are impaired in pregnancies after bariatric surgery due to the changed nutritional and  
12  
13 21 microbiome dynamics. Therefore, our aim is to conduct the Bariatrics and EmbrYONic Development  
14  
15 22 (BEYOND) study to investigate parameters of maternal nutritional and health status after bariatric  
16  
17 23 surgery, both periconceptionally and during pregnancy, particularly concentrating on embryonic and  
18  
19 24 foetal growth trajectories as well as placental development.  
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24 25 **Methods and analysis:** We designed a single-centre prospective, observational cohort, which  
25  
26 26 investigates the iatrogenic nutritional and health status of women after bariatric surgery,  
27  
28 27 periconceptionally and during pregnancy. The BEYOND study is embedded in the Rotterdam  
29  
30 28 Periconceptional Cohort, a tertiary hospital-based birth cohort study. Eligible participants are women  
31  
32 29 contemplating pregnancy or <12+0 weeks pregnant, ≥18 and ≤45 years of age, who have undergone  
33  
34 30 bariatric surgery (cases) or without prior bariatric surgery (controls) and their male partners. Medical  
35  
36 31 charts will be reviewed and questionnaires regarding general health, lifestyle and food intake will be  
37  
38 32 collected. Moreover, we will perform serial three-dimensional ultrasounds to assess embryonic  
39  
40 33 growth and placental development, and two-dimensional ultrasounds for foetal growth assessment.  
41  
42 34 The microbiome, including the virome, and blood samples will be sampled during the preconception  
43  
44 35 period and in each trimester. Multivariable linear mixed model analyses will be used to assess the  
45  
46 36 associations between bariatric surgery and pregnancy outcomes.  
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51 37 **Ethics and dissemination:** This proposal was approved by the Medical Ethics Committee from the  
52  
53 38 Erasmus MC, Rotterdam, The Netherlands). Study results will be submitted for publication in high-  
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55 39 impact journals, presented at scientific conferences, implemented into guidelines and communicated  
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3 40 through the Erasmus MC and collaborating partners.  
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5 41 **Trial registration number** NL8217 ([www.trialregister.nl](http://www.trialregister.nl)).  
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3 46 **Strengths and limitations of this study**  
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- 5 47 • **The investigation of embryonic and placental growth and development in post-bariatric**  
6 48 **patients will for the first time provide more insight into the underlying pathways and origins of**  
7 49 **the increased risk for foetal growth restriction and low birth weight in these pregnancies.**  
8 50 • **By prospective analysis of vitamin levels after vitamin supplement use in post-bariatric**  
9 51 **women, we will define reference values and tailored recommendations for vitamin**  
10 52 **supplementation for these women.**  
11 53 • **Due to ethical reasons, it is not possible to perform a randomised-controlled trial using**  
12 54 **bariatric surgery as intervention, as gold standard for clinical research.**  
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## 56 Introduction

57 The incidence of obesity, which is defined as a Body Mass Index (BMI) of  $\geq 30$  kg/m<sup>2</sup>, is increasing  
58 worldwide, including in women of reproductive age.<sup>1</sup> Importantly, prepregnancy and  
59 periconceptual obesity are now well-established independent risk factors for foetomaternal  
60 complications and disease risks in offspring as well as for women during the life course (e.g.  
61 hypertension and type II diabetes mellitus).<sup>2-5</sup> The risks along the life course can be significantly  
62 reduced by achieving prepregnancy weight loss and as such a reduction in BMI. Weight loss in  
63 obesity can be accomplished by life style and nutritional changes, bariatric surgery or a combination  
64 of both.

## 65 Bariatric surgery and related weight loss

66 Bariatric surgery is an effective surgical solution to quickly lose excess weight and reach a healthier  
67 long-term weight. Patients qualify for bariatric surgery if they have a BMI >40 kg/m<sup>2</sup>, or a BMI above  
68 35 kg/m<sup>2</sup> along with at least one obesity-related comorbidity such as diabetes mellitus.<sup>6</sup> There are  
69 three types of bariatric surgery: 1. malabsorptive surgery, in which the small intestines are partially  
70 bypassed, 2. restrictive surgery, in which the stomach size is decreased, and 3. a combination  
71 between malabsorptive and restrictive surgery. Since bariatric surgery leads to fast, excessive and -  
72 most importantly - long-term weight loss, preconceptional bariatric surgery in women of  
73 reproductive age can diminish the prevalence of obesity-related adverse maternal and foetal  
74 outcomes.<sup>7,8</sup> Unfortunately, due to fast and excessive weight loss resulting from gastro-intestinal  
75 anatomical changes of preconceptional bariatric surgery, iatrogenic malnutrition can also increase  
76 the incidence of adverse pregnancy and perinatal outcomes, such as intra-uterine growth restriction  
77 and congenital vitamin deficiencies in neonates.<sup>8-11</sup>

## 78 Bariatric surgery and nutritional status

79 Gastro-intestinal surgical changes after bariatric surgery can cause malabsorption and iatrogenic  
80 malnutrition, including vitamin deficiencies. A high incidence of vitamin deficiencies has been



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3 81 reported in patients after bariatric surgery.<sup>12</sup> Most reported deficiencies during the first trimester  
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5 82 after bariatric surgery are, amongst others, vitamin B1, folate, and vitamin D.<sup>13-17</sup> However, ample  
6  
7 83 research has been performed to map, treat and investigate consequences of these vitamin  
8  
9 84 deficiencies in these women during the periconception period, with potential consequences for  
10  
11 85 embryonic growth and development.<sup>18 19</sup> Vitamin B1 (thiamin) is needed for the synthesis of myelin  
12  
13 86 and involved in mitochondrial and synaptosomal membranes, and is vital for foetal neural and brain  
14  
15 87 development.<sup>20</sup> Vitamin B1 deficiency impacts intra-uterine growth, causing growth restriction,  
16  
17 88 while vitamin D deficiency can result in postnatal motor development disorders.<sup>21</sup> Folate deficiency  
18  
19 89 can lead to impaired oocyte quality, subfertility, congenital malformations, and several placenta-  
20  
21 90 related pregnancy complications.<sup>22-25</sup> Postsurgical multivitamin supplementation after bariatric  
22  
23 91 surgery is highly dosed to correct for the anticipated deficiencies and has only been developed for  
24  
25 92 the non-pregnant patient. Hereby, the used dosage regimen can lead to supraphysiological levels,  
26  
27 93 with potential teratogenic levels and detrimental effects for the developing foetus.<sup>26</sup>

#### 94 Nutritional status and mechanisms

95 An adequate maternal nutritional and vitamin status is essential for optimal foetal development.<sup>13 19-  
96 25</sup> Barker et al. were the first to suggest that maternal nutrition during pregnancy directs and  
97 programmes foetal development in utero, the so called “Developmental Origins of Health and  
98 Disease” dogma.<sup>27</sup> For example, maternal undernutrition has been associated with the susceptibility  
99 of developing non-communicable diseases in later life by foetal programming, whereas adequate  
100 nutritional health has been shown to reduce the odds of obesity in the offspring.<sup>28-30</sup>

#### 101 One-carbon metabolism and the microbiome

102 Obesity is also linked with an imbalanced one-carbon metabolism.<sup>31 32</sup> The one-carbon metabolism is  
103 essential for DNA methylation and gene expression and plays a vital role in physiological processes  
104 such as biosynthesis, cell division and proliferation.<sup>33 34</sup> Especially, embryonic and foetal growth are  
105 characterised by a need for rapid cellular multiplication, division and proliferation and therefore the

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3 106 one-carbon metabolism is crucial for embryonic and foetal development. Key players of the one-  
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5 107 carbon metabolism such as folate are either provided by diet, including vitamin supplements, or as  
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7 108 by-products of the bacterial metabolism. The gut microbiota, collectively known as the gut  
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9 109 microbiome, includes bacteria (bacteriome) as well as viruses (virome). Importantly, periconception  
10  
11 110 maternal nutritional status and alterations, such as bariatric surgery, can influence general cellular  
12  
13 111 function, metabolism and the gut microbiome. Bariatric surgery can hereby affect foetal growth and  
14  
15 112 metabolism. Bariatric surgery is besides anatomical gastro-intestinal tract changes also associated  
16  
17 113 with lifestyle and dramatic gut microbiome changes.<sup>35</sup> Therefore, current information is needed  
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19 114 regarding the influence of bariatric surgery on maternal nutritional status, the dynamics and  
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21 115 composition of the microbiome and one-carbon metabolism and their combined effect on  
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23 116 embryonic, foetal and placental development.  
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## 117 Methods and analysis

118 We designed a prospective single-centre observational cohort study, which is embedded in the  
119 Rotterdam Periconceptual cohort (Predict study, MEC 2004-227), to investigate post-bariatric  
120 women both before and during pregnancy with the aim to study the association between bariatric  
121 surgery and embryonic, foetal and maternal health. We used the SPIRIT checklist when writing our  
122 report.<sup>36</sup>

### 123 Study design (Figure 1)

#### 124 Setting

125 All study subjects will be included at the outpatient clinic of the Erasmus MC, University Medical  
126 Centre. Eligible cases will be provided with a study leaflet by health care professionals at the  
127 Bariatric Centre, Obstetric or Fertility outpatient clinic, after which they will be asked to  
128 participate. Women and their partners will be referred from the moment they are contemplating  
129 pregnancy and/or before 12+0 weeks gestation of pregnancy. Controls are selected from the Predict  
130 Study.<sup>37</sup> With the BEYOND study, serial embryonic and foetal growth parameters, the microbiome  
131 and blood samples will prospectively be documented. The primary objective of this study is to  
132 investigate the association between preconception maternal bariatric surgery and subsequent  
133 embryonic, foetal and placental growth. We hypothesise that bariatric surgery impairs embryonic  
134 and foetal growth due to postsurgical nutritional deficiencies. Secondary objectives include the  
135 investigation of associations between preconception bariatric surgery and clinical maternal and  
136 pregnancy outcomes, one-carbon metabolism effects such as homocysteine levels, the microbial  
137 composition, placental growth and development, and maternal lifestyle.

#### 138 Study population

139 In order to be eligible to participate in this study, women have to be  $\geq 18$  and  $\leq 45$  years of age, either  
140 contemplating pregnancy or  $\leq 12+0$  weeks pregnant of a singleton pregnancy. Their male partners will  
141 also be included. They must have sufficient understanding of the Dutch language and must be willing

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3 142 to give written informed consent. Cases must have had bariatric surgery, excluding a gastric banding  
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5 143 procedure that has been deflated or removed. Controls will be women who have not undergone  
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7 144 bariatric surgery and will be selected from the current Predict study.  
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#### 10 145 Study procedures

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13 146 The duration of the study will be 48 months and will include different measurements and procedures  
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16 147 (figure 1).  
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#### 19 148 Ultrasound scans

20  
21 149 At 7, 9, and 11 weeks gestational age (GA) the gestational sac, embryo, and placenta are  
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23 150 depicted in 3D ultrasound scans using a GE Voluson E8 or E10 Expert system with a 6-12  
24  
25 151 megahertz transvaginal probe and 4D View software (General Electric Medical Systems, Zipf,  
26  
27 152 Austria). We will perform 3D ultrasound scans that focus on embryonic and placental development  
28  
29 153 including crown rump length (CRL), embryonic volume (EV), brain structures and the gestational sac,  
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31 154 placenta and yolk sac. Moreover, we will evaluate the placental vasculature, pulsatility index and  
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33 155 resistance index of the uterine arteries at 9 and 11 weeks GA using ultrasound Doppler.  
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37 156 At 22-24 weeks GA and 30-32 weeks GA foetal growth will be assessed by the growth  
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39 157 parameters head circumference, biparietal diameter, abdominal circumference and femur  
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41 158 length. Moreover, ultrasound Dopplers of the umbilical arteries, middle cerebral artery and  
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43 159 uterine arteries will be performed.  
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47 160 All scans will be made with standard settings of the ultrasound machine: pulse repetition  
48  
49 161 frequency of 0.6 kilohertz, gain -2.0, quality "high", wall motion filter "low". The obtained 3D  
50  
51 162 datasets will be stored as Cartesian (rectangular) volumes. Moreover, we will follow the ALARA  
52  
53 163 (As Low As Reasonably Achievable) principle to ensure safe ultrasound examinations.  
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57 164 Pulsed wave Doppler is a standardised additional modality of ultrasound imaging to quantify  
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59 165 blood flow. Placental and uterine blood flow will be quantified as expressed by the resistance  
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3 166 index as well as by the pulsatility index. Hence, the use of pulsed wave Doppler signal during  
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5 167 ultrasound enables us to perform a non-invasive measurement of the blood flow and  
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7 168 subsequently to detect changes in flow.  
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#### 10 169 Questionnaires

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13 170 The women and their male partners will fill out a self-administered, validated questionnaire  
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15 171 regarding food intake of the previous four weeks.<sup>38-40</sup>  
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18 172 Moreover, preconceptionally and during the first trimester a general questionnaire will be filled  
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20 173 out including geographical background, education and lifestyle. At 24 weeks GA a questionnaire  
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22 174 is filled out regarding information about folic acid intake, lifestyle, prenatal screening, results of  
23  
24 175 the foetal anomaly scan (around 20 weeks of gestation) and previous pregnancy outcome.  
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26 176 Postpartum, women are asked to fill out a questionnaire regarding neonatal health. As follow-up  
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28 177 one year postpartum, women are asked to fill out the last questionnaire regarding congenital  
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30 178 malformations, general health and medical history of the offspring.  
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#### 33 179 Blood samples

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36 180 Preconceptionally and during all three trimesters of pregnancy, 2 blood samples of 10 mL in a  
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38 181 vacutainer ethylenediamine tetraacetate (EDTA) tube and 1 blood sample of 8.5 mL in a serum  
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40 182 tube will be drawn. These samples will be centrifuged directly and separated into serum,  
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42 183 plasma, whole blood and buffy coat aliquots. Parameters of the one-carbon metabolism such as  
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44 184 homocysteine serum levels will be measured.  
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48 185 Umbilical cord blood is collected at delivery in one EDTA tube (10 ml) and a separator tube (10  
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50 186 ml). After this process, all blood samples will be stored at -80 °C at the Erasmus MC for this  
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52 187 study and future research.  
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#### 55 188 Microbiome samples

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58 189 Preconceptionally and during all three trimesters of pregnancy, the vaginal and fecal  
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60 190 microbiome, including the virome, will be sampled. Vaginal and faecal samples will be self-

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3 191 collected by the patient. After collection, the vaginal and faecal samples will be stored at -80 °C  
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5 192 until processing. The microbiome samples will also be collected prospectively in the Predict  
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7 193 study cohort.  
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#### 10 194 Sample size

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13 195 We performed a simulation study to calculate the sample size and compared the profile of  
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15 196 embryonic volume in the simulation study with controls who had not undergone bariatric surgery  
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17 197 using a multivariate test. The relationship between bariatric surgery and the outcome could be  
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19 198 influenced by several confounders. In the sample size calculation, we focused on BMI and age of the  
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21 199 mother which are the most important confounders. In the control group, the mean of these variables  
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23 200 are respectively 25.5 kg/m<sup>2</sup> (SD=4.9 kg/m<sup>2</sup>) and 31.9 years (SD=4.5 years). In the bariatric surgery  
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25 201 group, we postulate that the mean BMI is 30.1 kg/m<sup>2</sup> at conception and the mean age is 31 years  
26  
27 202 with identical standard deviations as in the non-bariatric surgery group, based on recent research.<sup>14</sup>  
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29 203 <sup>16 41-49</sup> We aimed to detect a difference in the cube root embryonic volumes of 0.059 millimetres as  
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31 204 this is the difference between adequate and inadequate folate state.<sup>50</sup> The standard deviation of the  
32  
33 205 cube root embryonic volume given the covariates is 0.105. Simulations showed that we need at least  
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35 206 50 patients to detect a difference with 80% power and an alpha of 0.05. Given an average  
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37 207 miscarriage rate of 10% and an estimate of a 30% dropout, we will need to include 80 pregnant  
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39 208 patients. With an average chance to conceive within a year of 84% this results in a total number of 95  
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41 209 preconceptional patients.<sup>51</sup>  
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#### 47 210 Statistical analysis

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49 211 To assess the association between preconception bariatric surgery and embryonic growth  
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51 212 trajectories measured by embryonic volume, CRL and placental volume, we will perform  
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53 213 multivariable linear mixed model analyses using patients without bariatric surgery as a control group.  
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55 214 By using a mixed model, we consider a correlation between the observations within the same  
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57 215 pregnancy. Cube root transformation will be used to investigate if this results in linearity with GA and  
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3 216 is therefore a constant variant independent of GA. We will first perform a univariate analysis in which  
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5 217 we adjust for GA only. After this, we will enter all covariates that are significantly correlated with  
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7 218 bariatric surgery. The fully adjusted model will be made after stepwise elimination of all covariates  
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9 219 with p-values above the 20<sup>th</sup> percentile. Potential confounders will be identified by performing a  
10  
11 220 literature search, by using analysis of variance (ANOVA) and by calculating Spearman correlation  
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13 221 coefficients for the other maternal characteristics such as, but not limited to, maternal BMI, parity,  
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15 222 smoking, age, mode of conception and foetal sex.

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19 223 Missing data will be handled by multiple imputation. A p-value <0.05 will be considered as  
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21 224 statistically significant.

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25 225 The primary outcome parameter is defined as the embryonic growth trajectory assessed by serial  
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27 226 embryonic volumes. Since all other outcome parameters are secondary or descriptive no correction  
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29 227 for multiplicity will be performed. For categorical variables such as smoking and alcohol use we will  
30  
31 228 use descriptive characteristics. We will use mean and standard deviation for normally distributed  
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33 229 variables and median and interquartile range for not normally distributed variables.

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37 230 To test the influence of vitamin deficiencies and excesses related to supplement usage, distribution  
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39 231 will be tested using student's t-test for normal distributions, Kruskal-Wallis test for non-parametric  
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41 232 distributions and chi-squared or exact tests for categorical data.

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44 233 To assess the relationship between maternal parameters such as pregnancy outcome, bariatric  
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46 234 surgery and longitudinal changes in vaginal and faecal microbiome and blood samples, we will test if  
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48 235 the data are normally distributed by using the Shapiro-Wilks normality test. The independent  
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50 236 samples t-test and Mann-Whitney U test will be used for continuous data.

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54 237 Continuous, normally distributed variables will be presented as mean with standard deviation, and  
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56 238 variables with a skewed distribution as median with the range. Categorical variables will be  
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58 239 presented as count and proportions. Differences between women who have undergone bariatric  
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3 240 surgery and women who have not undergone bariatric surgery will be compared, correcting for  
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5 241 differences in BMI at conception.  
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8 242 To assess the association between preconception bariatric surgery and embryonic growth  
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10 243 trajectories by CRL, we will perform multivariable linear mixed model analyses. By using a mixed  
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12 244 model, we consider a correlation between the observations are within the same pregnancy.  
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16 245 We will repeat analyses in the subgroups of IVF/ICSI pregnancies only and in subgroups of different  
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18 246 types of bariatric surgery if the groups are large enough. Birth weight will be compared between  
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20 247 groups by taking GA at birth into account using linear regression analysis.  
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#### 23 248 **Data statement**

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26 249 The current study will abide by the principles of the Declaration of Helsinki (October 2013) and all  
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28 250 national and EU guidelines. The study protocol has been approved by the local Medical Ethics  
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30 251 Committee of the Erasmus Medical Centre (MEC 2019-0518) and has been registered in the Dutch  
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32 252 Trial Register (NL8217) ([www.trialregister.nl](http://www.trialregister.nl)). All participants will only be included after informed  
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34 253 consent.  
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37 254 Data will be pseudo-anonymised in order to guarantee the privacy of the patients by assigning a  
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39 255 study-ID. We will keep a strictly confidential mapping from their local patient-ID to the study-ID.  
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42 256 Patient data will be stored on a separate protected research storage platform. Access will be limited  
43  
44 257 to authorised medical personnel. All research data will be retained and stored in the study-database  
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46 258 at the Erasmus MC.  
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#### 49 259 **Patient and public involvement**

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52 260 Patients were not involved in the development of the research question, outcome measures or study  
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54 261 design, but the project will be communicated to patients by using the internal and external  
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56 262 communication means of the Erasmus MC, University Medical Centre, collaborating partners, such as  
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3 263 institutional and patient association websites, social media, magazines and the yearly news letter  
4  
5 264 from the Rotterdam periconception cohort.  
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8 265 **Ethics and dissemination**  
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10  
11 266 This study was prospectively registered in December 2019 after ethical approval by the Medical  
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13 267 Ethics Committee from the Erasmus MC, Rotterdam, The Netherlands. We will disseminate our study  
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15 268 results by publishing papers in high-impact journals, presentations at scientific conferences and  
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18 269 implement the results into local and (inter)national guidelines.  
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3 270 **Research implications**  
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5 271 The results of this study will provide detailed information about embryonic, foetal and placental  
6  
7 272 growth and development during pregnancy in post-bariatric women. Furthermore, this study will  
8  
9 273 elucidate maternal periconception health and pregnancy course, embryonic and foetal growth  
10  
11 274 trajectories and placental development in women after bariatric surgery. We also aim to gain more  
12  
13 275 insight into the underlying pathways leading to iatrogenic malnutrition after bariatric surgery and the  
14  
15 276 resulting weight loss, how these circumstances influence the local microbiome and how we could  
16  
17 277 potentially prevent them by timely correction. Particularly, it is of utmost importance to investigate  
18  
19 278 the effects on embryonic, foetal and placental development. With the results of the BEYOND study,  
20  
21 279 severely obese women contemplating bariatric surgery and pregnancy can be counselled more  
22  
23 280 precisely concerning the (dis)advantages of bariatric surgery related to the periconception period,  
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25 281 pregnancy and placental, embryonic, foetal and neonatal health. Therefore, improved  
26  
27 282 periconception health care counselling can be provided, allowing for better informed decision  
28  
29 283 making about bariatric surgery and a future pregnancy.  
30  
31 284 In general, we can equip health care providers with patient-tailored advice for obese women of  
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33 285 reproductive age during the periconception period. By analysing vitamin ranges from the  
34  
35 286 preconception period and all pregnancy trimester, we will be able to map both the incidence of  
36  
37 287 vitamin deficiencies and excesses due to (over)correction of deficiencies and supraphysiological  
38  
39 288 vitamin supplementation. Hence, we intend to gain insight into possible teratogenicity of high dose  
40  
41 289 vitamin supplementation. The results of our analyses will give insight into the incidence of vitamin  
42  
43 290 deficiencies and excesses after vitamin supplementation in post-bariatric women, and will allow us to  
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45 291 provide personalized counselling regarding optimal vitamin supplementation for these women.  
46  
47 292 Therefore, periconception health can be improved, resulting in improved foetal, placental, neonatal  
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49 293 and maternal health along the life course.  
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295 **References**

- 296 1. Finucane MM, Stevens GA, Cowan MJ, et al. National, regional, and global trends in body-mass  
297 index since 1980: systematic analysis of health examination surveys and epidemiological  
298 studies with 960 country-years and 9.1 million participants. *The Lancet* 2011;**377**(9765):557-  
299 67.
- 300 2. Athukorala C, Rumbold AR, Willson KJ, et al. The risk of adverse pregnancy outcomes in women  
301 who are overweight or obese. *BMC Pregnancy Childbirth* 2010;**10**:56.
- 302 3. Avci ME, Sanlikan F, Celik M, et al. Effects of maternal obesity on antenatal, perinatal and neonatal  
303 outcomes. *J Matern Fetal Neonatal Med* 2015;**28**(17):2080-3.
- 304 4. LifeCycle Project-Maternal O, Childhood Outcomes Study G, Voerman E, et al. Association of  
305 Gestational Weight Gain With Adverse Maternal and Infant Outcomes. *Jama*  
306 2019;**321**(17):1702-15.
- 307 5. Mission JF, Marshall NE, Caughey AB. Pregnancy Risks Associated with Obesity. *Obstetrics and*  
308 *Gynecology Clinics of North America* 2015;**42**(2):335-53.
- 309 6. NICE. CG43 obesity: NICE guideline. London: NICE 2010:pp 1-84.
- 310 7. Patel JA, Patel NA, Thomas RL, et al. Pregnancy outcomes after laparoscopic Roux-en-Y gastric  
311 bypass. *Surgery for Obesity and Related Diseases* 2008;**4**(1):39-45.
- 312 8. Belogolovkin V, Salihi HM, Weldeselasse H, et al. Impact of prior bariatric surgery on maternal and  
313 fetal outcomes among obese and non-obese mothers. *Archives of Gynecology and Obstetrics*  
314 2012;**285**(5):1211-18.
- 315 9. Cools M, Duval ELIM, Jaspers A. Adverse neonatal outcome after maternal biliopancreatic  
316 diversion operation: report of nine cases. *European Journal of Pediatrics* 2006;**165**(3):199-  
317 202.
- 318 10. Josefsson A, Blomberg M, Bladh M, et al. Bariatric surgery in a national cohort of women:  
319 sociodemographics and obstetric outcomes. *American Journal of Obstetrics and Gynecology*  
320 2011;**205**(3):206. e1-06. e8.
- 321 11. Lesko J, Peaceman A. Pregnancy outcomes in women after bariatric surgery compared with obese  
322 and morbidly obese controls. *Obstetrics & Gynecology* 2012;**119**(3):547-54.
- 323 12. Swinburn BA, Kraak VI, Allender S, et al. The global syndemic of obesity, undernutrition, and  
324 climate change: the Lancet Commission report. *The Lancet* 2019;**393**(10173):791-846.
- 325 13. Bebbler FE, Rizzolli J, Casagrande DS, et al. Pregnancy after bariatric surgery: 39 Pregnancies  
326 follow-up in a multidisciplinary team. *Obes Surg* 2011;**21**(10):1546-51.
- 327 14. Costa MM, Belo S, Souteiro P, et al. Pregnancy after bariatric surgery: Maternal and fetal  
328 outcomes of 39 pregnancies and a literature review. *J Obstet Gynaecol Res* 2018;**44**(4):681-  
329 90.
- 330 15. Gadgil MD, Chang HY, Richards TM, et al. Laboratory testing for and diagnosis of nutritional  
331 deficiencies in pregnancy before and after bariatric surgery. *J Women's Health*  
332 2014;**23**(2):129-37.
- 333 16. Hazart J, Le Guennec D, Accoceberry M, et al. Maternal Nutritional Deficiencies and Small-for-  
334 Gestational-Age Neonates at Birth of Women Who Have Undergone Bariatric Surgery. *J*  
335 *Pregnancy* 2017;**2017**:4168541.
- 336 17. Medeiros M, Matos AC, Pereira SE, et al. Vitamin D and its relation with ionic calcium,  
337 parathyroid hormone, maternal and neonatal characteristics in pregnancy after roux-en-Y  
338 gastric bypass. *Arch Gynecol Obstet* 2016;**293**(3):539-47.
- 339 18. Bebbler FE, Rizzolli J, Casagrande DS, et al. Pregnancy after Bariatric Surgery: 39 Pregnancies  
340 Follow-up in a Multidisciplinary Team. *Obesity Surgery* 2011;**21**(10):1546-51.
- 341 19. Devlieger R, Guelinckx I, Jans G, et al. Micronutrient levels and supplement intake in pregnancy  
342 after bariatric surgery: a prospective cohort study. *PLoS One* 2014;**9**(12):e114192.
- 343 20. Kloss O, Eskin NAM, Suh M. Thiamin deficiency on fetal brain development with and without  
344 prenatal alcohol exposure. *Biochemistry and Cell Biology* 2017;**96**(2):169-77.

- 1  
2  
3 345 21. Janbek J, Specht IO, Heitmann BL. Associations between vitamin D status in pregnancy and  
4 346 offspring neurodevelopment: a systematic literature review. *Nutr Rev* 2019.
- 5 347 22. Hague WM. Homocysteine and pregnancy. *Best Pract Res Clin Obstet Gynaecol* 2003;**17**(3):459-  
6 348 69.
- 7 349 23. Steegers-Theunissen RPM, Steegers EAP. Nutrient-gene interactions in early pregnancy: a  
8 350 vascular hypothesis: Elsevier, 2003.
- 9 351 24. Ebisch IMW, Thomas CMG, Peters WHM, et al. The importance of folate, zinc and antioxidants in  
10 352 the pathogenesis and prevention of subfertility. *Human Reproduction Update*  
11 353 2006;**13**(2):163-74.
- 12 354 25. Picciano MF. Is homocysteine a biomarker for identifying women at risk of complications and  
13 355 adverse pregnancy outcomes?: Oxford University Press, 2000.
- 14 356 26. Roth DE. Vitamin D supplementation during pregnancy: safety considerations in the design and  
15 357 interpretation of clinical trials. *Journal of Perinatology* 2011;**31**(7):449-59.
- 16 358 27. Hales CN, Barker DJ, Clark PM, et al. Fetal and infant growth and impaired glucose tolerance at  
17 359 age 64. *British Medical Journal* 1991;**303**(6809):1019-22.
- 18 360 28. Dhana K, Haines J, Liu G, et al. Association between maternal adherence to healthy lifestyle  
19 361 practices and risk of obesity in offspring: results from two prospective cohort studies of  
20 362 mother-child pairs in the United States. *Bmj* 2018;**362**:k2486.
- 21 363 29. Dhana K, Zong G, Yuan C, et al. Lifestyle of women before pregnancy and the risk of offspring  
22 364 obesity during childhood through early adulthood. *Int J Obes (Lond)* 2018;**42**(7):1275-84.
- 23 365 30. Barker DJ. The fetal and infant origins of adult disease. *BMJ: British Medical Journal*  
24 366 1990;**301**(6761):1111.
- 25 367 31. Ley RE, Bäckhed F, Turnbaugh P, et al. Obesity alters gut microbial ecology. *Proceedings of the*  
26 368 *national academy of sciences* 2005;**102**(31):11070-75.
- 27 369 32. Yadav H, Jain S, Nagpal R, et al. Increased fecal viral content associated with obesity in mice.  
28 370 *World journal of diabetes* 2016;**7**(15):316.
- 29 371 33. Knight AK, Park HJ, Hausman DB, et al. Association between one-carbon metabolism indices and  
30 372 DNA methylation status in maternal and cord blood. *Scientific Reports* 2018;**8**(1):1-9.
- 31 373 34. Ducker GS, Rabinowitz JD. One-carbon metabolism in health and disease. *Cell Metabolism*  
32 374 2017;**25**(1):27-42.
- 33 375 35. Farin W, Oñate FP, Plassais J, et al. Impact of laparoscopic Roux-en-Y gastric bypass and sleeve  
34 376 gastrectomy on gut microbiota: a metagenomic comparative analysis. *Surg Obes Relat Dis*  
35 377 2020;**16**(7):852-62.
- 36 378 36. Chan A-W, Tetzlaff JM, Gøtzsche PC, et al. SPIRIT 2013 explanation and elaboration: guidance for  
37 379 protocols of clinical trials. *Bmj* 2013;**346**.
- 38 380 37. Steegers-Theunissen RP, Verheijden-Paulissen JJ, van Uiter EM, et al. Cohort Profile: The  
39 381 Rotterdam Periconceptional Cohort (Predict Study). *Int J Epidemiol* 2016;**45**(2):374-81.
- 40 382 38. Thompson FE, Byers T. Dietary assessment resource manual. *The Journal of nutrition*  
41 383 1994;**124**(suppl\_11):2245s-317s.
- 42 384 39. Cade J, Thompson R, Burley V, et al. Development, validation and utilisation of food-frequency  
43 385 questionnaires—a review. *Public health nutrition* 2002;**5**(4):567-87.
- 44 386 40. Steinemann N, Grize L, Ziesemer K, et al. Relative validation of a food frequency questionnaire to  
45 387 estimate food intake in an adult population. *Food & Nutrition Research* 2017;**61**(1):1305193.
- 46 388 41. Basbug A, Ellibeş Kaya A, Dogan S, et al. Does pregnancy interval after laparoscopic sleeve  
47 389 gastrectomy affect maternal and perinatal outcomes? *J Matern -Fetal Neonatal Med* 2018:1-  
48 390 7.
- 49 391 42. Coupaye M, Legardeur H, Sami O, et al. Impact of Roux-en-Y gastric bypass and sleeve  
50 392 gastrectomy on fetal growth and relationship with maternal nutritional status. *Surg Obes*  
51 393 *Relat Dis* 2018.
- 52 394 43. De Carolis S, Botta A, Del Sordo G, et al. Influence of Biliopancreatic Diversion on Pregnancy  
53 395 Outcomes in Comparison to Other Bariatric Surgery Procedures. *Obes Surg*  
54 396 2018;**28**(10):3284-92.

- 1  
2  
3 397 44. Nilsson-Condori E, Hedenbro JL, Thurin-Kjellberg A, et al. Impact of diet and bariatric surgery on  
4 398 anti-Müllerian hormone levels. *Hum Reprod* 2018;**33**(4):690-93.  
5 399 45. Parent B, Martopullo I, Weiss NS, et al. Bariatric surgery in women of childbearing age, timing  
6 400 between an operation and birth, and associated perinatal complications. *JAMA Surg*  
7 401 2017;**152**(2):128-35.  
8 402 46. Rasteiro C, Araújo C, Cunha S, et al. Influence of Time Interval from Bariatric Surgery to  
9 403 Conception on Pregnancy and Perinatal Outcomes. *Obes Surg* 2018;**28**(11):3559-66.  
10 404 47. Sahab Al kabbi M, Al-Tae HA, Kareem Al Hussaini S. Impact of Bariatric surgery on antimullerian  
11 405 hormone in reproductive age women. *Middle East Fertil Soc J* 2018.  
12 406 48. Vincentelli C, Maraninchi M, Valéro R, et al. One-year impact of bariatric surgery on serum anti-  
13 407 Mullerian-hormone levels in severely obese women. *J Assisted Reprod Genet*  
14 408 2018;**35**(7):1317-24.  
15 409 49. Yau PO, Parikh M, Saunders JK, et al. Pregnancy after bariatric surgery: the effect of time-to-  
16 410 conception on pregnancy outcomes. *Surg Obes Relat Dis* 2017;**13**(11):1899-905.  
17 411 50. Van Dijk MR, Borggreven NV, Willemsen SP, et al. Maternal Lifestyle Impairs Embryonic Growth:  
18 412 The Rotterdam Periconception Cohort. *Reprod Sci* 2018;**25**(6):916-22.  
19 413 51. Andersen A-MN, Wohlfahrt J, Christens P, et al. Maternal age and fetal loss: population based  
20 414 register linkage study. *Bmj* 2000;**320**(7251):1708-12.  
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3 417 **Author statement**  
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5 418 KS, SS and RS designed the study. KS will be executing the research with  
6  
7 419 supervision from SS, RS and JL. RK is involved in the retrospective part of this study. RS is the  
8  
9 420 principal investigator in this study and SS is the principal coordinator. All authors have read and  
10  
11 421 approved the final manuscript.  
12  
13  
14

15 422 **Funding statement:**  
16

17  
18 423 Not applicable.  
19  
20

21 424 **Conflict of interests**  
22

23  
24 425 All authors declare no competing interests.  
25  
26

27 426 **Data availability**  
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30 427 Data are available upon reasonable request.  
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429 Figure 1. Enrolment, collection of data and materials and follow-up.

For peer review only

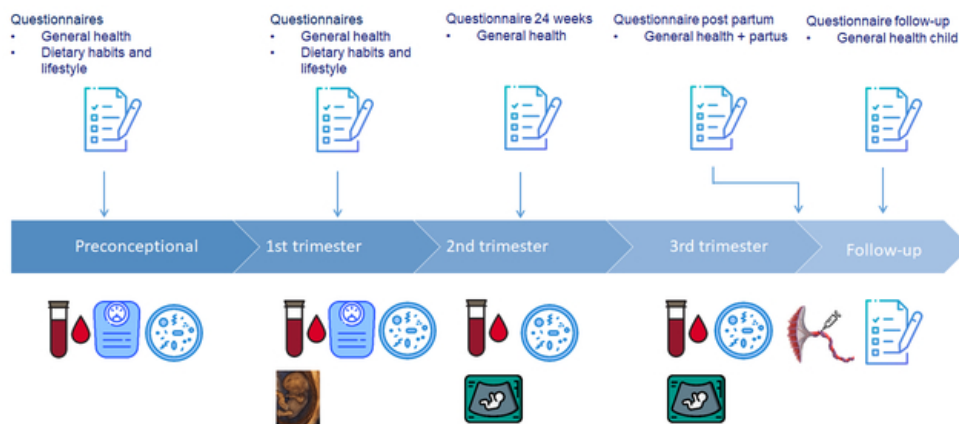


Figure 1.

27x11mm (600 x 600 DPI)



# Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. *BMJ*. 2013;346:e7586

	Reporting Item	Page Number
<b>Administrative information</b>		
Title	<a href="#">#1</a> Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

1	Trial registration	<a href="#">#2a</a>	Trial identifier and registry name. If not yet	3
2			registered, name of intended registry	
3				
4				
5				
6	Trial registration:	<a href="#">#2b</a>	All items from the World Health Organization Trial	n/a
7	data set		Registration Data Set	
8				
9				
10				
11				
12	Protocol version	<a href="#">#3</a>	Date and version identifier	n/a
13				
14				
15	Funding	<a href="#">#4</a>	Sources and types of financial, material, and other	19
16			support	
17				
18				
19				
20	Roles and	<a href="#">#5a</a>	Names, affiliations, and roles of protocol contributors	19
21	responsibilities:			
22				
23	contributorship			
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27				
28	Roles and	<a href="#">#5b</a>	Name and contact information for the trial sponsor	n/a
29	responsibilities:			
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31	sponsor contact			
32	information			
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38	Roles and	<a href="#">#5c</a>	Role of study sponsor and funders, if any, in study	n/a
39	responsibilities:		design; collection, management, analysis, and	
40			interpretation of data; writing of the report; and the	
41	sponsor and funder		decision to submit the report for publication,	
42			including whether they will have ultimate authority	
43			over any of these activities	
44				
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52	Roles and	<a href="#">#5d</a>	Composition, roles, and responsibilities of the	14
53	responsibilities:		coordinating centre, steering committee, endpoint	
54			adjudication committee, data management team,	
55	committees			
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and other individuals or groups overseeing the trial,  
if applicable (see Item 21a for data monitoring  
committee)

## Introduction

Background and rationale	<a href="#">#6a</a>	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5,6,7
Background and rationale: choice of comparators	<a href="#">#6b</a>	Explanation for choice of comparators	n/a
Objectives	<a href="#">#7</a>	Specific objectives or hypotheses	7,8
Trial design	<a href="#">#8</a>	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
<b>Methods:</b>			
<b>Participants, interventions, and outcomes</b>			
Study setting	<a href="#">#9</a>	Description of study settings (eg, community clinic, academic hospital) and list of countries where data	8

1		will be collected. Reference to where list of study	
2		sites can be obtained	
3			
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6	Eligibility criteria	<a href="#">#10</a> Inclusion and exclusion criteria for participants. If	8,9
7		applicable, eligibility criteria for study centres and	
8		individuals who will perform the interventions (eg,	
9		surgeons, psychotherapists)	
10			
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16	Interventions:	<a href="#">#11a</a> Interventions for each group with sufficient detail to	8,9,10,11
17		allow replication, including how and when they will	
18	description	be administered	
19			
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23	Interventions:	<a href="#">#11b</a> Criteria for discontinuing or modifying allocated	n/a
24		interventions for a given trial participant (eg, drug	
25	modifications	dose change in response to harms, participant	
26		request, or improving / worsening disease)	
27			
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33	Interventions:	<a href="#">#11c</a> Strategies to improve adherence to intervention	n/a
34		protocols, and any procedures for monitoring	
35	adherence	adherence (eg, drug tablet return; laboratory tests)	
36			
37			
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40			
41	Interventions:	<a href="#">#11d</a> Relevant concomitant care and interventions that are	n/a
42		permitted or prohibited during the trial	
43	concomitant care		
44			
45			
46	Outcomes	<a href="#">#12</a> Primary, secondary, and other outcomes, including	8
47		the specific measurement variable (eg, systolic	
48		blood pressure), analysis metric (eg, change from	
49		baseline, final value, time to event), method of	
50		aggregation (eg, median, proportion), and time point	
51		for each outcome. Explanation of the clinical	
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1 relevance of chosen efficacy and harm outcomes is  
 2  
 3 strongly recommended  
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5  
 6 Participant timeline [#13](#) Time schedule of enrolment, interventions (including 9, figure 1  
 7 any run-ins and washouts), assessments, and visits  
 8 for participants. A schematic diagram is highly  
 9 recommended (see Figure)  
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15  
 16 Sample size [#14](#) Estimated number of participants needed to achieve 11  
 17 study objectives and how it was determined,  
 18 including clinical and statistical assumptions  
 19 supporting any sample size calculations  
 20  
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25  
 26 Recruitment [#15](#) Strategies for achieving adequate participant Study protocol  
 27 enrolment to reach target sample size page 31,32  
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### 31 Methods:

### 32 33 Assignment of 34 35 interventions (for 36 37 controlled trials) 38 39 40

41 Allocation: sequence [#16a](#) Method of generating the allocation sequence (eg, n/a  
 42 generation computer-generated random numbers), and list of  
 43 any factors for stratification. To reduce predictability  
 44 of a random sequence, details of any planned  
 45 restriction (eg, blocking) should be provided in a  
 46 separate document that is unavailable to those who  
 47 enrol participants or assign interventions  
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1	Allocation	<a href="#">#16b</a>	Mechanism of implementing the allocation sequence	n/a
2				
3	concealment		(eg, central telephone; sequentially numbered,	
4				
5	mechanism		opaque, sealed envelopes), describing any steps to	
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8			conceal the sequence until interventions are	
9				
10			assigned	
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13	Allocation:	<a href="#">#16c</a>	Who will generate the allocation sequence, who will	n/a
14				
15	implementation		enrol participants, and who will assign participants to	
16				
17			interventions	
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21	Blinding (masking)	<a href="#">#17a</a>	Who will be blinded after assignment to interventions	n/a
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23			(eg, trial participants, care providers, outcome	
24				
25			assessors, data analysts), and how	
26				
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28	Blinding (masking):	<a href="#">#17b</a>	If blinded, circumstances under which unblinding is	n/a
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30	emergency		permissible, and procedure for revealing a	
31				
32	unblinding		participant's allocated intervention during the trial	
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36	<b>Methods: Data</b>			
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38	<b>collection,</b>			
39				
40	<b>management, and</b>			
41				
42	<b>analysis</b>			
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46	Data collection plan	<a href="#">#18a</a>	Plans for assessment and collection of outcome,	Study protocol
47				
48			baseline, and other trial data, including any related	page
49				
50			processes to promote data quality (eg, duplicate	33,34,35,36
51				
52			measurements, training of assessors) and a	
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54			description of study instruments (eg, questionnaires,	
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56			laboratory tests) along with their reliability and	
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1		validity, if known. Reference to where data collection	
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3		forms can be found, if not in the protocol	
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6	Data collection plan: <a href="#">#18b</a>	Plans to promote participant retention and complete	Study protocol
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8	retention	follow-up, including list of any outcome data to be	page
9			
10		collected for participants who discontinue or deviate	33,34,35,36
11			
12		from intervention protocols	
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16	Data management <a href="#">#19</a>	Plans for data entry, coding, security, and storage,	Study protocol
17			
18		including any related processes to promote data	page
19			
20		quality (eg, double data entry; range checks for data	33,34,35,36
21			
22		values). Reference to where details of data	
23			
24		management procedures can be found, if not in the	
25			
26		protocol	
27			
28			
29			
30	Statistics: outcomes <a href="#">#20a</a>	Statistical methods for analysing primary and	Study protocol
31			
32		secondary outcomes. Reference to where other	page 28,29,30
33			
34		details of the statistical analysis plan can be found, if	
35			
36		not in the protocol	
37			
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39			
40	Statistics: additional <a href="#">#20b</a>	Methods for any additional analyses (eg, subgroup	Study protocol
41			
42	analyses	and adjusted analyses)	page 28,29,30
43			
44			
45	Statistics: analysis <a href="#">#20c</a>	Definition of analysis population relating to protocol	Study protocol
46			
47	population and	non-adherence (eg, as randomised analysis), and	page 28
48			
49	missing data	any statistical methods to handle missing data (eg,	
50			
51		multiple imputation)	
52			
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54			
55	<b>Methods: Monitoring</b>		
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1	Data monitoring:	<a href="#">#21a</a>	Composition of data monitoring committee (DMC);	Study protocol
2				
3	formal committee		summary of its role and reporting structure;	page 36
4				
5			statement of whether it is independent from the	
6			sponsor and competing interests; and reference to	
7			where further details about its charter can be found,	
8			if not in the protocol. Alternatively, an explanation of	
9			why a DMC is not needed	
10				
11	Data monitoring:	<a href="#">#21b</a>	Description of any interim analyses and stopping	Study protocol
12				
13	interim analysis		guidelines, including who will have access to these	page 30
14			interim results and make the final decision to	
15			terminate the trial	
16				
17	Harms	<a href="#">#22</a>	Plans for collecting, assessing, reporting, and	Study protocol
18			managing solicited and spontaneously reported	page 26,27
19			adverse events and other unintended effects of trial	
20			interventions or trial conduct	
21				
22	Auditing	<a href="#">#23</a>	Frequency and procedures for auditing trial conduct,	26,27
23			if any, and whether the process will be independent	
24			from investigators and the sponsor	
25				
26	<b>Ethics and</b>			
27	<b>dissemination</b>			
28				
29	Research ethics	<a href="#">#24</a>	Plans for seeking research ethics committee /	Study protocol
30				
31	approval		institutional review board (REC / IRB) approval	page 32
32				
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1	Protocol	<a href="#">#25</a>	Plans for communicating important protocol	Study protocol
2				
3	amendments		modifications (eg, changes to eligibility criteria,	page 36
4			outcomes, analyses) to relevant parties (eg,	
5			investigators, REC / IRBs, trial participants, trial	
6			registries, journals, regulators)	
7				
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12				
13	Consent or assent	<a href="#">#26a</a>	Who will obtain informed consent or assent from	Study protocol
14			potential trial participants or authorised surrogates,	page 31,32
15			and how (see Item 32)	
16				
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18				
19				
20				
21	Consent or assent:	<a href="#">#26b</a>	Additional consent provisions for collection and use	n/a
22			of participant data and biological specimens in	
23	ancillary studies		ancillary studies, if applicable	
24				
25				
26				
27				
28	Confidentiality	<a href="#">#27</a>	How personal information about potential and	14
29			enrolled participants will be collected, shared, and	
30			maintained in order to protect confidentiality before,	
31			during, and after the trial	
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38	Declaration of	<a href="#">#28</a>	Financial and other competing interests for principal	19
39			investigators for the overall trial and each study site	
40	interests			
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43				
44	Data access	<a href="#">#29</a>	Statement of who will have access to the final trial	14
45			dataset, and disclosure of contractual agreements	
46			that limit such access for investigators	
47				
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51	Ancillary and post	<a href="#">#30</a>	Provisions, if any, for ancillary and post-trial care,	n/a
52			and for compensation to those who suffer harm from	
53	trial care		trial participation	
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1	Dissemination	<a href="#">#31a</a>	Plans for investigators and sponsor to communicate	14
2				
3	policy: trial results		trial results to participants, healthcare professionals,	
4			the public, and other relevant groups (eg, via	
5			publication, reporting in results databases, or other	
6			data sharing arrangements), including any	
7			publication restrictions	
8				
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10				
11				
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14				
15	Dissemination	<a href="#">#31b</a>	Authorship eligibility guidelines and any intended	n/a
16				
17	policy: authorship		use of professional writers	
18				
19				
20				
21	Dissemination	<a href="#">#31c</a>	Plans, if any, for granting public access to the full	n/a
22				
23	policy: reproducible		protocol, participant-level dataset, and statistical	
24			code	
25	research			
26				
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29	<b>Appendices</b>			
30				
31				
32	Informed consent	<a href="#">#32</a>	Model consent form and other related	n/a
33				
34	materials		documentation given to participants and authorised	
35			surrogates	
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39	Biological	<a href="#">#33</a>	Plans for collection, laboratory evaluation, and	n/a
40				
41	specimens		storage of biological specimens for genetic or	
42			molecular analysis in the current trial and for future	
43			use in ancillary studies, if applicable	
44				
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## Notes:

- 52 • 13: 9, figure 1
- 55 • 15: Study protocol page 31,32
- 58 • 18a: Study protocol page 33,34,35,36

- 1 • 18b: Study protocol page 33,34,35,36
- 2
- 3
- 4 • 19: Study protocol page 33,34,35,36
- 5
- 6
- 7 • 20a: Study protocol page 28,29,30
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- 9
- 10 • 20b: Study protocol page 28,29,30
- 11
- 12
- 13 • 20c: Study protocol 28
- 14
- 15
- 16 • 21a: Study protocol 36
- 17
- 18
- 19 • 21b: Study protocol 30
- 20
- 21
- 22 • 22: Study protocol page 26,27
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- 24
- 25 • 24: Study protocol page 32
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- 28 • 25: Study protocol page 36
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- 30
- 31 • 26a: Study protocol 31,32 The SPIRIT Explanation and Elaboration paper is distributed under the
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# BMJ Open

## The impact of Bariatric surgery on EmbrYONic, foetal and placental Development (BEYOND): Protocol for a prospective cohort study embedded in the Rotterdam periconception cohort

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-051110.R1
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<b>Primary Subject Heading</b>:	Obstetrics and gynaecology
Secondary Subject Heading:	Surgery
Keywords:	SURGERY, EPIDEMIOLOGY, NUTRITION & DIETETICS, OBSTETRICS, Maternal medicine < OBSTETRICS

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3 1 **The impact of Bariatric surgery on EmbrYONic, foetal and placental Development (BEYOND):**

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5 2 **Protocol for a prospective cohort study embedded in the Rotterdam periconception cohort**

6  
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32 12 **Keywords:**

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34 13 Surgery, epidemiology, nutrition&dietetics, obstetrics, maternal medicine

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37 14 **Word count:**

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39 15 2976

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3 16 **Abstract**  
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5 17 **Introduction:** The worldwide obesity epidemic has resulted in a rise of bariatric surgery in women of  
6  
7 18 reproductive age which can lead to 'iatrogenic undernutrition'. Long lasting undernutrition can affect  
8  
9 19 maternal health, pregnancy outcomes and offspring. We hypothesise that embryonic and placental  
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11 20 growth are impaired in pregnancies after bariatric surgery due to the changed nutritional and  
12  
13 21 microbiome dynamics. Therefore, our aim is to conduct the Bariatrics and EmbrYONic Development  
14  
15 22 (BEYOND) study to investigate parameters of maternal nutritional and health status after bariatric  
16  
17 23 surgery, both periconceptionally and during pregnancy, particularly concentrating on embryonic and  
18  
19 24 foetal growth trajectories as well as placental development.  
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23  
24 25 **Methods and analysis:** We designed a single-centre prospective, observational cohort, which  
25  
26 26 investigates the iatrogenic nutritional and health status of women after bariatric surgery,  
27  
28 27 periconceptionally and during pregnancy. The BEYOND study is embedded in the Rotterdam  
29  
30 28 Periconceptional Cohort, a tertiary hospital-based birth cohort study. Eligible participants are women  
31  
32 29 planning pregnancy or <12+0 weeks pregnant, ≥18 and ≤45 years of age, who have undergone  
33  
34 30 bariatric surgery (cases) or without prior bariatric surgery (controls) and their male partners. Medical  
35  
36 31 charts will be reviewed and questionnaires regarding general health, lifestyle and food intake will be  
37  
38 32 collected. Moreover, we will perform serial three-dimensional ultrasounds to assess embryonic  
39  
40 33 growth and placental development, and two-dimensional ultrasounds for foetal growth assessment.  
41  
42 34 The microbiome, including the virome, and blood samples will be sampled during the preconception  
43  
44 35 period and in each trimester. Multivariable linear mixed model analyses will be used to assess the  
45  
46 36 associations between bariatric surgery and pregnancy outcomes.  
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51 37 **Ethics and dissemination:** This proposal was approved by the Medical Ethics Committee from the  
52  
53 38 Erasmus MC, Rotterdam, The Netherlands. Study results will be submitted for publication in high-  
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55 39 impact journals, presented at scientific conferences, implemented into guidelines and communicated  
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3 40 through the Erasmus MC and collaborating partners.  
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5 41 **Trial registration number** NL8217 ([www.trialregister.nl](http://www.trialregister.nl)).  
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For peer review only



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3 46 **Strengths and limitations of this study**  
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5 47

- 6 48 • **The prospective and longitudinal assessment of postbariatric women preconceptionally, during**  
7 49 **and after pregnancy provides an in-depth knowledge of the reproductive trajectory of these**  
8 50 **women.**  
9 51 • **The prospective design of this study allows us to analyse vitamin levels after vitamin**  
10 52 **supplement use in postbariatric women, which gives the opportunity to define individualised**  
11 53 **and tailored recommendations for additional vitamin supplementation.**  
12 54 • **The three-dimensional ultrasound examinations during the first trimester provide detailed**  
13 55 **information regarding embryonic, foetal and placental growth and development in**  
14 56 **postbariatric women for the first time.**  
15 57 • **Due to ethical reasons, the design of this study is not a randomised-controlled trial, being the**  
16 58 **gold standard for clinical research.**  
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## 59 Introduction

60 The incidence of obesity, which is defined as a Body Mass Index (BMI) of  $\geq 30$  kg/m<sup>2</sup>, is increasing  
61 worldwide, including in women of reproductive age.<sup>1</sup> Importantly, prepregnancy and  
62 periconceptual obesity are now well-established independent risk factors for foetomaternal  
63 complications and disease risks in offspring as well as for women during the life course (e.g.  
64 hypertension and type II diabetes mellitus).<sup>2-5</sup> The risks along the life course can be significantly  
65 reduced by achieving prepregnancy weight loss and as such a reduction in BMI. Weight loss in  
66 obesity can be accomplished by lifestyle and nutritional changes, pharmaceutical therapies or  
67 medical weight loss interventions programs, bariatric surgery or a combination of these  
68 interventions.

## 69 Bariatric surgery and related weight loss

70 Bariatric surgery is an effective surgical solution to quickly lose excess weight and reach a healthier  
71 long-term weight.<sup>6-10</sup> Patients qualify for bariatric surgery if they have a BMI > 40 kg/m<sup>2</sup>, or a BMI  
72 above 35 kg/m<sup>2</sup> along with at least one obesity-related comorbidity such as diabetes mellitus.<sup>11-15</sup>  
73 There are different types of bariatric surgery, based on their endocrine, metabolic and  
74 (patho)physiological consequences. Malabsorptive procedures lead to impaired uptake of nutrients,  
75 whereas restrictive procedures mainly decrease food intake. However, a sleeve gastrectomy, which is  
76 often considered a restrictive procedure, also has endocrine and metabolic effects.<sup>16</sup> Since bariatric  
77 surgery leads to fast, excessive and - most importantly - long-term weight loss, preconceptional  
78 bariatric surgery in women of reproductive age can diminish the prevalence of obesity-related  
79 adverse maternal and foetal outcomes.<sup>17-21</sup> Unfortunately, due to fast and excessive weight loss  
80 resulting from gastro-intestinal anatomical changes of preconceptional bariatric surgery, iatrogenic  
81 malnutrition can also increase the incidence of adverse pregnancy and perinatal outcomes, such  
82 as intra-uterine growth restriction and congenital vitamin deficiencies in neonates. Growth  
83 restriction seems to be mainly present after malabsorptive surgery, as nutritional deficiencies occur  
84 more often after this type of bariatric procedure.<sup>18,19</sup>

### 85 Bariatric surgery and nutritional status

86 Gastro-intestinal surgical changes after bariatric surgery can cause malabsorption and iatrogenic  
87 malnutrition, including vitamin deficiencies. A high incidence of vitamin deficiencies has been  
88 reported in patients after bariatric surgery.<sup>22</sup> Most reported deficiencies during the first trimester  
89 after bariatric surgery are, amongst others, vitamin B1, folate, and vitamin D.<sup>23-26</sup> However, ample  
90 research has been performed to map, treat and investigate consequences of these vitamin  
91 deficiencies in these women during the periconception period, with potential consequences for  
92 embryonic growth and development.<sup>27 28</sup> Vitamin B1 (thiamin) is needed for the synthesis of myelin  
93 and involved in mitochondrial and synaptosomal membranes, and is vital for foetal neural and brain  
94 development.<sup>29</sup> Vitamin B1 deficiency impacts intra-uterine growth, causing growth restriction,  
95 while vitamin D deficiency can result in postnatal motor development disorders.<sup>30</sup> Folate deficiency  
96 can lead to impaired oocyte quality, subfertility, congenital malformations, and several placenta-  
97 related pregnancy complications.<sup>31-34</sup> Postsurgical multivitamin supplementation after bariatric  
98 surgery is highly dosed to correct for the anticipated deficiencies and has only been developed for  
99 the non-pregnant patient. Hereby, the used dosage regimen can lead to supraphysiological levels,  
100 with potential teratogenic levels and detrimental effects for the developing foetus.<sup>35</sup>

### 101 Nutritional status and mechanisms

102 An adequate maternal nutritional and vitamin status is essential for optimal foetal development.<sup>28-34</sup>  
103 <sup>36</sup> Barker et al. were the first to suggest that maternal nutrition during pregnancy directs and  
104 programmes foetal development in utero, the so called "Developmental Origins of Health and  
105 Disease" dogma.<sup>37</sup> For example, maternal undernutrition has been associated with the susceptibility  
106 of developing non-communicable diseases in later life by foetal programming, whereas adequate  
107 nutritional health has been shown to reduce the odds of obesity in the offspring.<sup>38-40</sup>

### 108 One-carbon metabolism and the microbiome

1  
2  
3 109 Obesity is also linked with an imbalanced one-carbon metabolism.<sup>41 42</sup> The one-carbon metabolism is  
4  
5 110 essential for DNA methylation and gene expression and plays a vital role in physiological processes  
6  
7 111 such as biosynthesis, cell division and proliferation.<sup>43 44</sup> Especially, embryonic and foetal growth are  
8  
9 112 characterised by a need for rapid cellular multiplication, division and proliferation and therefore the  
10  
11 113 one-carbon metabolism is crucial for embryonic and foetal development. Key players of the one-  
12  
13 114 carbon metabolism such as folate are either provided by diet, including vitamin supplements, or as  
14  
15 115 by-products of the bacterial metabolism. The gut microbiota, collectively known as the gut  
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17 116 microbiome, includes bacteria (bacteriome) as well as viruses (virome). Importantly, periconception  
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19 117 maternal nutritional status and alterations, such as bariatric surgery, can influence general cellular  
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21 118 function, metabolism and the gut microbiome. Bariatric surgery can hereby affect foetal growth and  
22  
23 119 metabolism. Bariatric surgery is besides anatomical gastro-intestinal tract changes also associated  
24  
25 120 with lifestyle and dramatic gut microbiome changes.<sup>45</sup> Therefore, current information is needed  
26  
27 121 regarding the influence of bariatric surgery on maternal nutritional status, the dynamics and  
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29 122 composition of the microbiome and one-carbon metabolism and their combined effect on  
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31 123 embryonic, foetal and placental development.  
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## 124 **Methods and analysis**

125 We designed a prospective single-centre observational cohort study, which is embedded in the  
126 Rotterdam Periconceptual cohort (Predict study, MEC 2004-227), to investigate postbariatric  
127 women both before and during pregnancy with the aim to study the association between bariatric  
128 surgery and embryonic, foetal and maternal health.

### 129 Study design

#### 130 Setting

131 All study subjects will be included at the outpatient clinic of the Erasmus MC, University Medical  
132 Centre. Eligible cases will be provided with a study leaflet by health care professionals at the  
133 Bariatric Centre, Obstetric or Fertility outpatient clinic, after which they will be asked to  
134 participate. Women and their partners will be referred from the moment they are planning  
135 pregnancy and/or before 12+0 weeks gestation of pregnancy. Controls are selected from the Predict  
136 Study.<sup>46</sup> With the BEYOND study, serial embryonic and foetal growth parameters, the microbiome  
137 and blood samples will prospectively be documented. The primary objective of this study is to  
138 investigate the association between preconception maternal bariatric surgery and subsequent  
139 embryonic, foetal and placental growth. We hypothesise that bariatric surgery impairs embryonic  
140 and foetal growth due to postsurgical nutritional deficiencies. Secondary objectives include the  
141 investigation of associations between preconception bariatric surgery and clinical maternal and  
142 pregnancy outcomes, one-carbon metabolism effects such as homocysteine levels, the microbial  
143 composition, placental growth and development, and maternal lifestyle.

#### 144 Study population

145 In order to be eligible to participate in this study, women have to be  $\geq 18$  and  $\leq 45$  years of age, either  
146 planning pregnancy or  $\leq 12+0$  weeks pregnant of a singleton pregnancy. Their male partners will also  
147 be included. They must have sufficient understanding of the Dutch language and must be willing to  
148 give written informed consent. Cases must have had bariatric surgery, excluding a gastric banding

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3 149 procedure that has been deflated or removed prior to pregnancy. Controls will be women who have  
4  
5 150 not undergone bariatric surgery and will be selected from the current Predict study.  
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8 151 Study procedures  
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11 152 The duration of the study will be 48 months and will include different measurements and procedures  
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13  
14 153 (figure 1).  
15

16 154 Ultrasound scans  
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18  
19 155 At 7, 9, and 11 weeks gestational age (GA) the gestational sac, embryo, and placenta are  
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21 156 depicted in 3D ultrasound scans using a GE Voluson E8 or E10 Expert system with a 6-12  
22  
23 157 megahertz transvaginal probe and 4D View software (General Electric Medical Systems, Zipf,  
24  
25 158 Austria). We will perform 3D ultrasound scans that focus on embryonic and placental development  
26  
27 159 including crown rump length (CRL), embryonic volume (EV), brain structures and the gestational sac,  
28  
29 160 placenta and yolk sac. Moreover, we will evaluate the placental vasculature, pulsatility index and  
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31 161 resistance index of the uterine arteries at 9 and 11 weeks GA using ultrasound Doppler.  
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35 162 At 22-24 weeks GA and 30-32 weeks GA foetal growth will be assessed by the growth  
36  
37 163 parameters head circumference, biparietal diameter, abdominal circumference and femur  
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39 164 length. Moreover, ultrasound Dopplers of the umbilical arteries, middle cerebral artery and  
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41 165 uterine arteries will be performed.  
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45 166 All scans will be made with standard settings of the ultrasound machine: pulse repetition  
46  
47 167 frequency of 0.6 kilohertz, gain -2.0, quality "high", wall motion filter "low". The obtained 3D  
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49 168 datasets will be stored as Cartesian (rectangular) volumes. Moreover, we will follow the ALARA  
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51 169 (As Low As Reasonably Achievable) principle to ensure safe ultrasound examinations.  
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55 170 Pulsed wave Doppler is a standardised additional modality of ultrasound imaging to quantify  
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57 171 blood flow. Placental and uterine blood flow will be quantified as expressed by the resistance  
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59 172 index as well as by the pulsatility index. Hence, the use of pulsed wave Doppler signal during  
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3 173 ultrasound enables us to perform a non-invasive measurement of the blood flow and  
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5 174 subsequently to detect changes in flow.  
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### 8 175 Questionnaires

9  
10 176 Women and their male partners will fill out a self-administered, validated food frequency  
11  
12 177 questionnaire regarding food intake of the previous four weeks.<sup>47-49</sup> Portion sizes are quantified  
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15 178 by this questionnaire.  
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18 179 Moreover, preconceptionally and during the first trimester a general questionnaire will be filled  
19  
20 180 out including geographical background, education and lifestyle. At 24 weeks GA a questionnaire  
21  
22 181 is filled out regarding information about folic acid intake, any vitamin supplementation, lifestyle  
23  
24 182 behaviour, prenatal screening, results of the foetal anomaly scan (around 20 weeks of gestation)  
25  
26 183 and previous pregnancy outcome. Postpartum, women are asked to fill out a questionnaire  
27  
28 184 regarding neonatal health. As follow-up one year postpartum, women are asked to fill out the  
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30 185 last questionnaire regarding congenital malformations, general health and medical history of the  
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32 186 offspring.  
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### 36 187 Blood samples

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39 188 Preconceptionally and during all three trimesters of pregnancy, 2 blood samples of 10 mL in a  
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41 189 vacutainer ethylenediamine tetraacetate (EDTA) tube and 1 blood sample of 8.5 mL in a serum  
42  
43 190 tube will be drawn. These samples will be centrifuged directly and separated into serum,  
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45 191 plasma, whole blood and buffy coat aliquots. Parameters of the one-carbon metabolism such as  
46  
47 192 homocysteine serum levels will be measured.  
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50 193 Umbilical cord blood is collected at delivery in one EDTA tube (10 ml) and a separator tube (10  
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52 194 ml). After this process, all blood samples will be stored at -80 °C at the Erasmus MC for this  
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54 195 study and future research.  
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### 57 196 Microbiome samples

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60 197 Preconceptionally and during all three trimesters of pregnancy, the vaginal and fecal

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3 198 microbiome, including the virome, will be sampled. Vaginal and faecal samples will be self-  
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5 199 collected by the patient. After collection, the vaginal and faecal samples will be stored at -80 °C  
6  
7 200 until processing. The microbiome samples will also be collected prospectively in the Predict  
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9 201 study cohort.  
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### 11 12 13 202 Sample size

14  
15 203 We performed a simulation study to calculate the sample size and compared the profile of  
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17 204 embryonic volume in the simulation study with controls who had not undergone bariatric surgery  
18  
19 205 using a multivariate test. The relationship between bariatric surgery and the outcome could be  
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21 206 influenced by several confounders. In the sample size calculation, we focused on BMI and age of the  
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23 207 mother which are the most important confounders. In the control group, the mean of these variables  
24  
25 208 are respectively 25.5 kg/m<sup>2</sup> (SD=4.9 kg/m<sup>2</sup>) and 31.9 years (SD=4.5 years). In the bariatric surgery  
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27 209 group, we postulate that the mean BMI is 30.1 kg/m<sup>2</sup> at conception and the mean age is 31 years  
28  
29 210 with identical standard deviations as in the non-bariatric surgery group, based on recent research.<sup>50</sup>  
30  
31 211 <sup>60</sup> We aimed to detect a difference in the cube root embryonic volumes of 0.059 millimetres as this is  
32  
33 212 the difference between adequate and inadequate folate state.<sup>61</sup> The standard deviation of the cube  
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35 213 root embryonic volume given the covariates is 0.105. Simulations showed that we need at least 50  
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37 214 patients to detect a difference with 80% power and an alpha of 0.05. Given an average miscarriage  
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39 215 rate of 10% and an estimate of a 30% dropout, we will need to include 80 pregnant patients. With an  
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41 216 average chance to conceive within a year of 84% this results in a total number of 95 preconceptional  
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43 217 patients.<sup>62</sup>  
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### 49 218 Statistical analysis

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51 219 To assess the association between preconception bariatric surgery and embryonic growth  
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53 220 trajectories measured by embryonic volume, CRL and placental volume, we will perform  
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55 221 multivariable linear mixed model analyses using patients without bariatric surgery as a control group.  
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57 222 By using a mixed model, we consider a correlation between the observations within the same  
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3 223 pregnancy. Cube root transformation will be used to investigate if this results in linearity with GA and  
4  
5 224 is therefore a constant variant independent of GA. We will first perform a univariate analysis in which  
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7 225 we adjust for GA only. After this, we will enter all covariates that are significantly correlated with  
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9 226 bariatric surgery. The fully adjusted model will be made after stepwise elimination of all covariates  
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11 227 with p-values above the 20<sup>th</sup> percentile. Potential confounders will be identified by performing a  
12  
13 228 literature search, by using analysis of variance (ANOVA) and by calculating Spearman correlation  
14  
15 229 coefficients for the other maternal characteristics such as, but not limited to, maternal BMI, parity,  
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17 230 smoking, age, mode of conception and foetal sex.

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21 231 Missing data will be handled by multiple imputation. A p-value <0.05 will be considered as  
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23 232 statistically significant.

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27 233 The primary outcome parameter is defined as the embryonic growth trajectory assessed by serial  
28  
29 234 embryonic volumes. Since all other outcome parameters are secondary or descriptive no correction  
30  
31 235 for multiplicity will be performed. For categorical variables such as smoking and alcohol use we will  
32  
33 236 use descriptive characteristics. We will use mean and standard deviation for normally distributed  
34  
35 237 variables and median and interquartile range for not normally distributed variables.

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39 238 To test the influence of vitamin deficiencies and excesses related to supplement usage, distribution  
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41 239 will be tested using student's t-test for normal distributions, Kruskal-Wallis test for non-parametric  
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43 240 distributions and chi-squared or exact tests for categorical data.

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47 241 To assess the relationship between maternal parameters such as pregnancy outcome, bariatric  
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49 242 surgery and longitudinal changes in vaginal and faecal microbiome and blood samples, we will test if  
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51 243 the data are normally distributed by using the Shapiro-Wilks normality test. The independent  
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53 244 samples t-test and Mann-Whitney U test will be used for continuous data.

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57 245 Continuous, normally distributed variables will be presented as mean with standard deviation, and  
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59 246 variables with a skewed distribution as median with the range. Categorical variables will be

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3 247 presented as count and proportions. Differences between women who have undergone bariatric  
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5 248 surgery and women who have not undergone bariatric surgery will be compared, correcting for  
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7 249 differences in BMI at conception.  
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10 250 To assess the association between preconception bariatric surgery and embryonic growth  
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12 251 trajectories by CRL, we will perform multivariable linear mixed model analyses. By using a mixed  
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14 252 model, we consider a correlation between the observations are within the same pregnancy.  
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18 253 We will repeat analyses in the subgroups of IVF/ICSI pregnancies only and in subgroups of different  
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20 254 types of bariatric surgery if the groups are large enough. Birth weight will be compared between  
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22 255 groups by taking GA at birth into account using linear regression analysis.  
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#### 25 256 **Data statement**

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28 257 The current study will abide by the principles of the Declaration of Helsinki (October 2013) and all  
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30 258 national and EU guidelines. The study protocol has been approved by the local Medical Ethics  
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32 259 Committee of the Erasmus Medical Centre (MEC 2019-0518) and has been registered in the Dutch  
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34 260 Trial Register (NL8217) ([www.trialregister.nl](http://www.trialregister.nl)). All participants will only be included after informed  
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36 261 consent.  
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40 262 Data will be pseudo-anonymised in order to guarantee the privacy of the patients by assigning a  
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42 263 study-ID. We will keep a strictly confidential mapping from their local patient-ID to the study-ID.  
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44 264 Patient data will be stored on a separate protected research storage platform. Access will be limited  
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46 265 to authorised medical personnel. All research data will be retained and stored in the study-database  
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48 266 at the Erasmus MC.  
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#### 51 267 **Patient and public involvement**

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55 268 Patients were not involved in the development of the research question, outcome measures or study  
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57 269 design, but the project will be communicated to patients by using the internal and external  
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59 270 communication means of the Erasmus MC, University Medical Centre, collaborating partners, such as  
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3 271 institutional and patient association websites, social media, magazines and the yearly news letter  
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5 272 from the Rotterdam periconception cohort.  
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8 **273 Ethics and dissemination**  
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11 274 This study was prospectively registered in December 2019 after ethical approval by the Medical  
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13 275 Ethics Committee from the Erasmus MC, Rotterdam, The Netherlands. We will disseminate our study  
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15  
16 276 results by publishing papers in high-impact journals, presentations at scientific conferences and  
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18 277 implement the results into local and (inter)national guidelines.  
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3 278 **Research implications**  
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5 279 The results of this study will provide detailed information about embryonic, foetal and placental  
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7 280 growth and development during pregnancy in postbariatric women. Furthermore, this study will  
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9 281 elucidate maternal periconception health and pregnancy course, embryonic and foetal growth  
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11 282 trajectories and placental development in women after bariatric surgery. We also aim to gain more  
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13 283 insight into the underlying pathways leading to iatrogenic malnutrition after bariatric surgery and the  
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15 284 resulting weight loss, how these circumstances influence the local microbiome and how we could  
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17 285 potentially prevent them by timely correction. Particularly, it is of utmost importance to investigate  
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19 286 the effects on embryonic, foetal and placental development. With the results of the BEYOND study,  
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21 287 severely obese women planning bariatric surgery and pregnancy can be counselled more precisely  
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23 288 concerning the (dis)advantages of bariatric surgery related to the periconception period, pregnancy  
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25 289 and placental, embryonic, foetal and neonatal health. Therefore, improved periconception health  
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27 290 care counselling can be provided, allowing for better informed decision making about bariatric  
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29 291 surgery and a future pregnancy.  
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34 292 In general, we can equip health care providers with patient-tailored advice for obese women of  
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36 293 reproductive age during the periconception period. By analysing vitamin ranges from the  
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38 294 preconception period and all pregnancy trimester, we will be able to map both the incidence of  
39  
40 295 vitamin deficiencies and excesses due to (over)correction of deficiencies and supraphysiological  
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42 296 vitamin supplementation. Hence, we intend to gain insight into possible teratogenicity of high dose  
43  
44 297 vitamin supplementation. Besides, quantitative and qualitative information about dietary intake will  
45  
46 298 be retrieved from the food frequency questionnaires and will provide insight into possible dietary  
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48 299 issues in these patients. This can contribute to health care improvement delivered by dietitians and  
49  
50 300 other health care professionals for postbariatric pregnant patients. The results of our analyses will  
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52 301 give insight into the incidence of vitamin deficiencies and excesses after vitamin supplementation in  
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54 302 postbariatric women, and will allow us to provide personalized counselling regarding optimal vitamin  
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303 supplementation for these women. Therefore, periconception health can be improved, resulting in  
304 improved foetal, placental, neonatal and maternal health along the life course.

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For peer review only

306 **References**

- 307 1. Finucane MM, Stevens GA, Cowan MJ, et al. National, regional, and global trends in body-mass  
308 index since 1980: systematic analysis of health examination surveys and epidemiological  
309 studies with 960 country-years and 9.1 million participants. *The Lancet* 2011;**377**(9765):557-  
310 67.
- 311 2. Athukorala C, Rumbold AR, Willson KJ, et al. The risk of adverse pregnancy outcomes in women  
312 who are overweight or obese. *BMC Pregnancy Childbirth* 2010;**10**:56.
- 313 3. Avci ME, Sanlikan F, Celik M, et al. Effects of maternal obesity on antenatal, perinatal and neonatal  
314 outcomes. *J Matern Fetal Neonatal Med* 2015;**28**(17):2080-3.
- 315 4. LifeCycle Project-Maternal O, Childhood Outcomes Study G, Voerman E, et al. Association of  
316 Gestational Weight Gain With Adverse Maternal and Infant Outcomes. *Jama*  
317 2019;**321**(17):1702-15.
- 318 5. Mission JF, Marshall NE, Caughey AB. Pregnancy Risks Associated with Obesity. *Obstetrics and*  
319 *Gynecology Clinics of North America* 2015;**42**(2):335-53.
- 320 6. Colquitt JL, Pickett K, Loveman E, et al. Surgery for weight loss in adults. *Cochrane Database of*  
321 *Systematic Reviews* 2014(8).
- 322 7. Chang S-H, Stoll CRT, Song J, et al. The Effectiveness and Risks of Bariatric Surgery: An Updated  
323 Systematic Review and Meta-analysis, 2003-2012. *Jama Surgery* 2014;**149**(3):275-87.
- 324 8. Maciejewski ML, Arterburn DE, Van Scoyoc L, et al. Bariatric Surgery and Long-term Durability of  
325 Weight Loss. *Jama Surgery* 2016;**151**(11):1046-55.
- 326 9. Wolfe BM, Kvach E, Eckel RH. Treatment of obesity: weight loss and bariatric surgery. *Circulation*  
327 research 2016;**118**(11):1844-55.
- 328 10. Dumon KR, Murayama KM. Bariatric surgery outcomes. *Surgical Clinics* 2011;**91**(6):1313-38.
- 329 11. Heelkunde NVv. Chirurgische behandeling van obesitas: Federatie Medisch Specialisten; 2020  
330 [updated 28/10/2020. Available from:  
331 [https://richtlijndatabase.nl/richtlijn/chirurgische\\_behandeling\\_van\\_obesitas/startpagina\\_-](https://richtlijndatabase.nl/richtlijn/chirurgische_behandeling_van_obesitas/startpagina_-_chirurgische_behandeling_van_obesitas.html)  
332 [chirurgische\\_behandeling\\_van\\_obesitas.html](https://richtlijndatabase.nl/richtlijn/chirurgische_behandeling_van_obesitas.html).
- 333 12. Fried M, Hainer V, Basdevant A, et al. Interdisciplinary European guidelines on surgery of severe  
334 obesity. *Obesity Facts* 2008;**1**(1):52-59.
- 335 13. Mechanick JI, Youdim A, Jones DB. AACE/TOS/ASSMBS Guidelines: Clinical practice guideline for  
336 the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery  
337 patient-2013 update: cosponsored by American Association of Clinical Endocrinologists, the  
338 obesity society, and American society for metabolic & bariatric surgery. *Endocrine Practice*  
339 2013;**19**(2).
- 340 14. Ridley N. Expert panel on weight loss surgery-executive report. *Obes Res* 2005;**13**:206-26.
- 341 15. Fried M, Yumuk V, Oppert JM, et al. Interdisciplinary European Guidelines on Metabolic and  
342 Bariatric Surgery. *Obesity Surgery* 2014;**24**(1):42-55.
- 343 16. Benaiges D, Más-Lorenzo A, Goday A, et al. Laparoscopic sleeve gastrectomy: more than a  
344 restrictive bariatric surgery procedure? *World Journal of Gastroenterology*  
345 2015;**21**(41):11804.
- 346 17. Maggard MA, Yermilov I, Li Z, et al. Pregnancy and fertility following bariatric surgery: A  
347 systematic review. *J Am Med Assoc* 2008;**300**(19):2286-96.
- 348 18. Kwong W, Tomlinson G, Feig DS. Maternal and neonatal outcomes after bariatric surgery; a  
349 systematic review and meta-analysis: do the benefits outweigh the risks? *American Journal*  
350 *of Obstetrics and Gynecology* 2018;**218**(6):573-80.
- 351 19. Akhter Z, Rankin J, Ceulemans D, et al. Pregnancy after bariatric surgery and adverse perinatal  
352 outcomes: A systematic review and meta-analysis. *Plos Medicine* 2019;**16**(8):e1002866.
- 353 20. Shawe J, Ceulemans D, Akhter Z, et al. Pregnancy after bariatric surgery: Consensus  
354 recommendations for periconception, antenatal and postnatal care. *Obesity Reviews*  
355 2019;**20**(11):1507-22.

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60

21. Al-Nimr RI, Hakeem R, Moreschi JM, et al. Effects of bariatric surgery on maternal and infant outcomes of pregnancy—an evidence analysis center systematic review. *Journal of the Academy of Nutrition and Dietetics* 2019;**119**(11):1921-43.
22. Bloomberg RD, Fleishman A, Nalle JE, et al. Nutritional deficiencies following bariatric surgery: what have we learned? *Obesity Surgery* 2005;**15**(2):145-54.
23. Davies DJ, Baxter JM, Baxter JN. Nutritional deficiencies after bariatric surgery. *Obesity Surgery* 2007;**17**(9):1150-58.
24. Bal BS, Finelli FC, Shope TR, et al. Nutritional deficiencies after bariatric surgery. *Nature Reviews Endocrinology* 2012;**8**(9):544-56.
25. Xanthakos SA. Nutritional deficiencies in obesity and after bariatric surgery. *Pediatric Clinics* 2009;**56**(5):1105-21.
26. Falcone V, Stopp T, Feichtinger M, et al. Pregnancy after bariatric surgery: a narrative literature review and discussion of impact on pregnancy management and outcome. *BMC pregnancy and childbirth* 2018;**18**(1):1-13.
27. Bebbler FE, Rizzolli J, Casagrande DS, et al. Pregnancy after Bariatric Surgery: 39 Pregnancies Follow-up in a Multidisciplinary Team. *Obesity Surgery* 2011;**21**(10):1546-51.
28. Devlieger R, Guelinckx I, Jans G, et al. Micronutrient levels and supplement intake in pregnancy after bariatric surgery: a prospective cohort study. *PLoS One* 2014;**9**(12):e114192.
29. Kloss O, Eskin NAM, Suh M. Thiamin deficiency on fetal brain development with and without prenatal alcohol exposure. *Biochemistry and Cell Biology* 2017;**96**(2):169-77.
30. Janbek J, Specht IO, Heitmann BL. Associations between vitamin D status in pregnancy and offspring neurodevelopment: a systematic literature review. *Nutr Rev* 2019.
31. Hague WM. Homocysteine and pregnancy. *Best Pract Res Clin Obstet Gynaecol* 2003;**17**(3):459-69.
32. Steegers-Theunissen RPM, Steegers EAP. Nutrient-gene interactions in early pregnancy: a vascular hypothesis: Elsevier, 2003.
33. Ebisch IMW, Thomas CMG, Peters WHM, et al. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. *Human Reproduction Update* 2006;**13**(2):163-74.
34. Picciano MF. Is homocysteine a biomarker for identifying women at risk of complications and adverse pregnancy outcomes?: Oxford University Press, 2000.
35. Roth DE. Vitamin D supplementation during pregnancy: safety considerations in the design and interpretation of clinical trials. *Journal of Perinatology* 2011;**31**(7):449-59.
36. Bebbler FE, Rizzolli J, Casagrande DS, et al. Pregnancy after bariatric surgery: 39 Pregnancies follow-up in a multidisciplinary team. *Obes Surg* 2011;**21**(10):1546-51.
37. Hales CN, Barker DJ, Clark PM, et al. Fetal and infant growth and impaired glucose tolerance at age 64. *British Medical Journal* 1991;**303**(6809):1019-22.
38. Dhana K, Haines J, Liu G, et al. Association between maternal adherence to healthy lifestyle practices and risk of obesity in offspring: results from two prospective cohort studies of mother-child pairs in the United States. *Bmj* 2018;**362**:k2486.
39. Dhana K, Zong G, Yuan C, et al. Lifestyle of women before pregnancy and the risk of offspring obesity during childhood through early adulthood. *Int J Obes (Lond)* 2018;**42**(7):1275-84.
40. Barker DJ. The fetal and infant origins of adult disease. *BMJ: British Medical Journal* 1990;**301**(6761):1111.
41. Ley RE, Bäckhed F, Turnbaugh P, et al. Obesity alters gut microbial ecology. *Proceedings of the national academy of sciences* 2005;**102**(31):11070-75.
42. Yadav H, Jain S, Nagpal R, et al. Increased fecal viral content associated with obesity in mice. *World journal of diabetes* 2016;**7**(15):316.
43. Knight AK, Park HJ, Hausman DB, et al. Association between one-carbon metabolism indices and DNA methylation status in maternal and cord blood. *Scientific Reports* 2018;**8**(1):1-9.
44. Ducker GS, Rabinowitz JD. One-carbon metabolism in health and disease. *Cell Metabolism* 2017;**25**(1):27-42.

- 1  
2  
3 408 45. Farin W, Oñate FP, Plassais J, et al. Impact of laparoscopic Roux-en-Y gastric bypass and sleeve  
4 409 gastrectomy on gut microbiota: a metagenomic comparative analysis. *Surg Obes Relat Dis*  
5 410 2020;**16**(7):852-62.
- 6 411 46. Steegers-Theunissen RP, Verheijden-Paulissen JJ, van Uitert EM, et al. Cohort Profile: The  
7 412 Rotterdam Periconceptional Cohort (Predict Study). *Int J Epidemiol* 2016;**45**(2):374-81.
- 8 413 47. Thompson FE, Byers T. Dietary assessment resource manual. *The Journal of nutrition*  
9 414 1994;**124**(suppl\_11):2245s-317s.
- 10 415 48. Cade J, Thompson R, Burley V, et al. Development, validation and utilisation of food-frequency  
11 416 questionnaires—a review. *Public health nutrition* 2002;**5**(4):567-87.
- 12 417 49. Steinemann N, Grize L, Ziesemer K, et al. Relative validation of a food frequency questionnaire to  
13 418 estimate food intake in an adult population. *Food & Nutrition Research* 2017;**61**(1):1305193.
- 14 419 50. Basbug A, Ellibeş Kaya A, Dogan S, et al. Does pregnancy interval after laparoscopic sleeve  
15 420 gastrectomy affect maternal and perinatal outcomes? *J Matern -Fetal Neonatal Med* 2018:1-  
16 421 7.
- 17 422 51. Costa MM, Belo S, Souteiro P, et al. Pregnancy after bariatric surgery: Maternal and fetal  
18 423 outcomes of 39 pregnancies and a literature review. *J Obstet Gynaecol Res* 2018;**44**(4):681-  
19 424 90.
- 20 425 52. Coupaye M, Legardeur H, Sami O, et al. Impact of Roux-en-Y gastric bypass and sleeve  
21 426 gastrectomy on fetal growth and relationship with maternal nutritional status. *Surg Obes*  
22 427 *Relat Dis* 2018.
- 23 428 53. De Carolis S, Botta A, Del Sordo G, et al. Influence of Biliopancreatic Diversion on Pregnancy  
24 429 Outcomes in Comparison to Other Bariatric Surgery Procedures. *Obes Surg*  
25 430 2018;**28**(10):3284-92.
- 26 431 54. Hazart J, Le Guennec D, Accoceberry M, et al. Maternal Nutritional Deficiencies and Small-for-  
27 432 Gestational-Age Neonates at Birth of Women Who Have Undergone Bariatric Surgery. *J*  
28 433 *Pregnancy* 2017;**2017**:4168541.
- 29 434 55. Nilsson-Condori E, Hedenbro JL, Thurin-Kjellberg A, et al. Impact of diet and bariatric surgery on  
30 435 anti-Müllerian hormone levels. *Hum Reprod* 2018;**33**(4):690-93.
- 31 436 56. Parent B, Martopullo I, Weiss NS, et al. Bariatric surgery in women of childbearing age, timing  
32 437 between an operation and birth, and associated perinatal complications. *JAMA Surg*  
33 438 2017;**152**(2):128-35.
- 34 439 57. Rasteiro C, Araújo C, Cunha S, et al. Influence of Time Interval from Bariatric Surgery to  
35 440 Conception on Pregnancy and Perinatal Outcomes. *Obes Surg* 2018;**28**(11):3559-66.
- 36 441 58. Sahab Al kabbi M, Al-Tae HA, Kareem Al Hussaini S. Impact of Bariatric surgery on antimüllerian  
37 442 hormone in reproductive age women. *Middle East Fertil Soc J* 2018.
- 38 443 59. Vincentelli C, Maraninchi M, Valéro R, et al. One-year impact of bariatric surgery on serum anti-  
39 444 Müllerian-hormone levels in severely obese women. *J Assisted Reprod Genet*  
40 445 2018;**35**(7):1317-24.
- 41 446 60. Yau PO, Parikh M, Saunders JK, et al. Pregnancy after bariatric surgery: the effect of time-to-  
42 447 conception on pregnancy outcomes. *Surg Obes Relat Dis* 2017;**13**(11):1899-905.
- 43 448 61. Van Dijk MR, Borggrevén NV, Willemsen SP, et al. Maternal Lifestyle Impairs Embryonic Growth:  
44 449 The Rotterdam Periconception Cohort. *Reprod Sci* 2018;**25**(6):916-22.
- 45 450 62. Andersen A-MN, Wohlfahrt J, Christens P, et al. Maternal age and fetal loss: population based  
46 451 register linkage study. *Bmj* 2000;**320**(7251):1708-12.

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3 454 **Author statement**  
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5 455 KS, SS and RS designed the study. KS will be executing the research with  
6  
7 456 supervision from SS, RS and JL. RK is involved in the retrospective part of this study. RS is the  
8  
9 457 principal investigator in this study and SS is the principal coordinator. All authors have read and  
10  
11 approved the final manuscript.  
12 458  
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15 459 **Funding statement:**  
16

17  
18 460 Not applicable.  
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21 461 **Conflict of interests**  
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23  
24 462 All authors declare no competing interests.  
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27 463 **Data availability**  
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30 464 Data are available upon reasonable request.  
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3 466 Figure 1. Enrolment, collection of data and materials and follow-up.  
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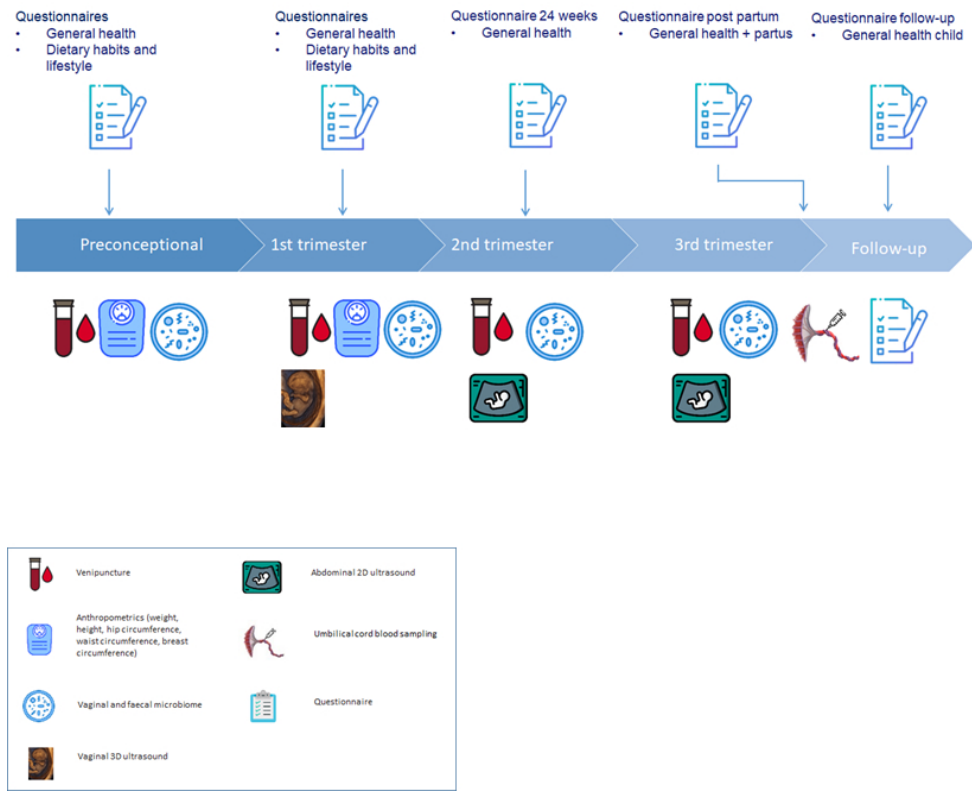


Figure 1

71x56mm (300 x 300 DPI)