The PROMOTE study, a pilot: The characterization of the microbiome in pregnancy and prediction of pregnancy outcomes.

Version 2.2

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PROTOCOL TITLE 'The PROMOTE study, a pilot: The characterization of the microbiome in pregnancy and prediction of pregnancy outcomes.

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Independent expert (s)	Marijn Vermeulen
Laboratory sites	Clinical chemistry Laboratory Erasmus MC
Pharmacy	Not applicable

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
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Coordinating Investigator/Project leader: Dr. S. Schoenmakers		
Principal Investigator: Prof. dr. R.P.M. Steegers-Theunissen		

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
CV	**Curriculum Vitae**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
IC	**Informed Consent**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
(S)AE	**(Serious) Adverse Event**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
GDM	**Gestational Diabetes Mellitus**
BMI	**Body Mass Index**

SUMMARY

Rationale: Our research aims to elucidate an underlying mechanism of maternal obesity (MOB)-related pregnancy and long-term health complications for mothers and their offspring. With the rising global prevalence of obesity, MOB-related pregnancy problems are increasingly occurring. Microbial gut symbiosis plays an important role in health, where dysbiosis is associated with diseases as obesity. Of interest, pregnancy, dietary patterns and pre- or probiotics affect the composition of the gut microbiome. The microbiome itself can influence many physiological processes, such as immune responses (production of microbial products) and the nutrient dependent one-carbon metabolism. We hypothesize that gut dysbiosis in MOB can be regarded as an endogenous chronic stressor inducing deranged immune responses and one-carbon metabolism. Both processes result in excessive oxidative stress, detrimental for cell multiplication, differentiation and epigenetic programming of maternal and offspring tissues. Together, these biological derangements contribute to placental and vascular dysfunction resulting in increased risks of preeclampsia or gestational diabetes mellitus. Vertical (during pregnancy) and horizontal (during delivery) transfer of gut dysbiosis from mother to new-born, and epigenetic placental and fetal changes can ultimately accumulate in macrosomia and childhood obesity.

Objective:
Research question: Is maternal microbiome dysbiosis in MOB an underlying mechanism in the pathophysiology of adverse maternal pregnancy and offspring outcome?

Objectives:
Analyse the differences between the gut and vaginal microbiome, maternal and fetal immune response and one-carbon metabolism in normal weight and obese pregnant women.

Study design: A single center prospective longitudinal observational cohort pilot study embedded in the Rotterdam periconception cohort (Predict study, METC 2004-227) will be performed periconceptional/early in the first trimester and continue until the delivery/postpartum at the Erasmus MC.

Study population: The Predict study population includes all pregnant women > 18 years and < 45 years who are willing to participate. Two participant groups will be included: 50 (pregnant) women with a BMI > 30 kg/m^2 (cases) and 50 (pregnant) women with a BMI: > 18 and < 25 kg/m^2 (controls) and their neonates (n = 100). In addition, 10 preconceptional

women with BMI > 30 kg/m^2 and 10 preconceptional women with BMI 18-25 kg/m^2 will be included. Making a total of 220 participants.

Intervention: Not applicable.

Main study parameters/endpoints: The composition of the maternal gut and vaginal microbiome (in obese women (BMI >30 kg/m2) and non-obese women (BMI 18-25 kg/m2)).

Secondary study parameters: The associations between the composition of the microbiome and:

1) Clinical maternal outcomes (e.g. pregnancy outcome, gestational age at delivery, preeclampsia, hypertension, gestational diabetes)
2) Clinical fetal outcomes (e.g. fetal growth trajectories in first, second and third trimester, birthweight)
3) Maternal (and fetal) immune response and one-carbon metabolism (e.g. inflammatory and biomarkers)
4) Placental function (e.g. placental vascular architecture and volume during the 1st trimester, placental weight, inflammatory status)

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: For all cases, the risks involve primarily the burden of participating in a study, which usually means additional hospital visits and assessments. There will be a maximum of 6 hospital visits, which will take approximately 30-45 minutes each. There will be two/three first trimester vaginal ultrasound examinations, two abdominal ultrasound examinations during the second and third trimester and 1 postpartum visit (all part of the Predict study, METC 2004-227). At a maximum of 5 of the appointments (periconceptional, in the first, second, third trimester, postpartum) blood draws and rectal swabs will be obtained. The first blood draw is combined with the blood draw of the Predict study, the three/four consecutive blood draws are additional for the PROMOTE study, as are the rectal swabs. Maternal anthropometrics, including weight, height and blood pressure will be measured preconceptionally or during the first trimester (as part of the Predict study). The risks of participation are considered to be nil and the potential benefit outweighs the risks. From the above explanations, it is clear that there are no obvious risks associated with participation in the study. After each visit, the participant will receive a picture of their fetus obtained by 3D or 2D ultrasound, if it is possible to generate one.

1. INTRODUCTION AND RATIONALE

Our research aims to elucidate the underlying mechanisms of maternal obesity (MOB)-related pregnancy and long-term health complications for mothers and their offspring. In Europe around 16,5% of all people is obese (BMI > 30 kg/m2), in the Netherlands this is percentage is around 15%, and the region of Rotterdam around 18%-20% (RIVM, 2020). With a total of ~170.000 pregnancies in the Netherlands in 2020 (RIVM), and one in every 5-6 pregnant woman being obese, this results in 25.000-35.000 MOB related pregnancies per year in the Dutch reproductive population (1,2). MOB is a significant risk factor for maternal complications, such as gestational diabetes mellitus (GDM), preeclampsia (3) and adverse birth outcomes, such as macrosomia with neonatal obesity extending in 2/3 of these babies into later life (4,5). MOB thus has a devastating impact on health, quality of life and healthcare costs (6-8). In order to break the vicious cycle of MOB, we will investigate the impact of derangements of the maternal gut microbiome (dysbiosis) in MOB women, during the periconceptional period, pregnancy and delivery as an underlying mechanism of the pregnancy and fetal complications.

Microbial gut symbiosis plays an important role in health (9), whereas dysbiosis is associated with diseases as obesity (10) and can be induced by an unhealthy diet, lifestyle, toxins or medication (11). Interestingly, also pregnancy (12,13) and pre- or probiotics (14) affect the gut microbiome. The microbiome itself can influence many physiological processes, such as immune responses (for instance by production of microbial products) (15) and the nutrient dependent one-carbon metabolism (supply of substrates and cofactors by the microbiota) (16,22). Obesity is associated with gut dysbiosis, also during pregnancy (10). The first evidence for a causal link between obesity and dysbiosis was derived from transplanting intestinal microbiome from obese mice into germfree mice resulting in replication of the disrupted obese metabolism in germfree mice (19). Similar results were obtained by transplanting human obese faeces to germfree mice (20). Moreover, faecal microbiota from a lean human donor to an obese human host resulted in decreased host insulin sensitivity (21). In addition, obesity is associated with derangement of the immune response (f.i. general low-grade inflammation) and in the one-carbon metabolism (22,23).

We therefore hypothesize that gut dysbiosis in MOB is an endogenous chronic stressor inducing deranged immune responses and one-carbon metabolism. Both processes result in excessive oxidative stress, detrimental for cell multiplication, differentiation and epigenetic programming of maternal and fetal tissues (24,25). Together, these biological derangements contribute to placental and vascular dysfunction resulting in increased risks of preeclampsia (26) or GDM (27). Although, gut colonization mainly starts at delivery by horizontal transfer of

the maternal vaginal and faecal microbiome to the new-born (28), during pregnancy, microbial products are already transferred to the fetus (29). Hence, maternal dysbiosis in MOB will be passed to the fetus and new-born, with adverse consequences for the offspring gut microbiome. The transfer of dysbiosis, together with adverse fetal and placental epigenetic changes may explain the health repercussion of MOB for offspring in later life, i.e., obesity “inheritance”. Our hypothesis of the role of gut dysbiosis in MOB is substantiated by various studies showing that treatment with prebiotics, such as fructooligosaccharides (FOS) and galactooligosaccharides (GOS), or probiotics (Lactobacillus, Bifidobacterium) stimulate the restoration of the microbiome resulting in an improved immune responses and one-carbon metabolism (30) and decreased oxidative stress (31). In addition, these pre-/probiotics reduce bodyweight and fat mass in obese mice and humans (32), whereas animal studies show that these treatments also have positive effects on the offspring (33,34). From this background it is our ultimate goal to contribute with this pilot study to better understanding, treatment and prevention of MOB-related maternal and fetal complications.

2. OBJECTIVES

Research question:
Is maternal microbiome dysbiosis in MOB an underlying mechanism in the pathophysiology of adverse maternal pregnancy and offspring outcome?

Objective:
1) Analyse the differences between the gut microbiome, maternal and fetal immune response and one-carbon metabolism in obese vs normal weight pregnant women.

3. STUDY DESIGN

A single center prospective longitudinal observational pilot cohort study, embedded in the Rotterdam periconception cohort (Predict study, METC 2004-227) will be performed periconceptional/early in the first trimester, during pregnancy and postpartum, at the Erasmus MC. The setting will be mainly at the outpatient clinic, partly at the delivery ward and partly at home. The study schedule is planned for the period of 48 months.

4. STUDY POPULATION

4.1 Population

This pilot study is embedded in the Rotterdam Periconceptional Cohort study (Predict study), a study that was set up in 2004 and is still ongoing. In total, 2846 women were

included up until June 2021 (35). The Predict study population includes preconceptional or pregnant women > 18 years and < 45 years old visiting the Erasmus MC and that are willing to participate. We will select the participants, that meet our undermentioned inclusion criteria, for our study from this cohort: 50 women with a BMI > 30 kg/m^2 (cases) and 50 women with a BMI ranging 18-25 kg/m^2 (controls) and their neonates.
Given the fact, that the number of inclusions per year in the Predict study is around 235 it is likely that we will achieve our sample size. We will longitudinally sample first/2nd/3rd/postpartum, 100 women, in addition we will include 10 preconceptional women with BMI > 30 kg/m^2 and 10 preconceptional women with BMI 18-25 kg/m^2, these participants do not necessarily have to conceive a pregnancy in order to remain in the study (these women are part of the preconceptional Predict population).

4.2 Inclusion criteria

For eligibility to participate in this study, a subject must meet all of the following criteria:

- Participation in Predict study
- Preconceptional women who wish to become pregnant or pregnancy <13 weeks of gestational age.
- BMI > 30 kg/m^2 or 18-25 kg/m^2
- Understanding of Dutch in speaking and reading
- Willingness to give written informed consent

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Age < 18 years and > 45 years.
- ≥13 weeks of gestational age
- Multiple pregnancy
- Smoking
- Gastro-intestinal diseases, heart diseases, liver, pancreas and kidney diseases.
- Use of antibiotics < 2 weeks before sampling
- Pre-existent diabetes mellitus
- Unable or unwilling to give informed consent

4.4 Sample size calculation

Since this is a pilot study, exploratory power calculations are not appropriate at this stage. We will include 50 (pregnant) women with a BMI > 30 kg/m^2 (cases) and 50 (pregnant) women with a BMI 18-25 kg/m^2 (controls) and their neonates (*n* =100). And 20

preconceptional women with BMI of 18-25 kg/m^2 (*n*=10) and BMI > 30 kg/m^2 (*n*=10). Furthermore, performing a power calculation would require prior estimates of all parameters in a model explaining the relation between the measurements and the outcomes, which is the goal of this study.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Not applicable.

5.2 Use of co-intervention

Not applicable.

5.3 Escape medication

Not applicable.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

Not applicable.

6.2 Summary of findings from non-clinical studies

Not applicable.

6.3 Summary of findings from clinical studies

Not applicable.

6.4 Summary of known and potential risks and benefits

Not applicable.

6.5 Description and justification of route of administration and dosage

Not applicable.

6.6 Dosages, dosage modifications and method of administration

Not applicable.

6.7 Preparation and labelling of Investigational Medicinal Product

Not applicable.

6.8 Drug accountability

Not applicable.

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

Not applicable.

7.2 Summary of findings from non-clinical studies

Not applicable.

7.3 Summary of findings from clinical studies

Not applicable.

7.4 Summary of known and potential risks and benefits

Not applicable.

7.5 Description and justification of route of administration and dosage

Not applicable.

7.6 Dosages, dosage modifications and method of administration

Not applicable.

7.7 Preparation and labelling of Non Investigational Medicinal Product

Not applicable.

7.8 Drug accountability

Not applicable.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The composition of the maternal gut microbiome in obese women (BMI >30 kg/m2) and non-obese women (BMI 18-25 kg/m2) during the preconceptional period, pregnancy and postpartum period.

8.1.2 Secondary study parameters/endpoints

The associations between the composition of the microbiome and:

1) Clinical maternal outcomes (e.g. pregnancy outcome gestational age at delivery, preeclampsia, hypertension, gestational diabetes)
2) Maternal conditions and lifestyle (e.g. diet, medication use, working activities, physical activity, intoxications)

3) Clinical fetal outcomes (e.g. growth trajectories in first, second and third trimester, birthweight)
4) Maternal (and fetal) immune response and one-carbon metabolism (e.g. inflammatory and biomarkers)
5) Placental function (e.g. placental weight, inflammatory status)

8.1.3 Other study parameters

Not applicable

8.2 Randomisation, blinding and treatment allocation

Not applicable

8.3 Study procedures

This paragraph describes the investigations and procedures that participants will undergo in the detailed scheme followed by an extensive description of procedures.

8.3.1 Detailed schedule of visits and assessment of the study:

Preconceptional	1st trimester	2nd & 3rd trimester	Delivery	Postpartum
Optional (n=20)				
1 visit	2-3 visits: 7,9,11th week GA	1 visit: 22-24th and 30-32th week GA		>8 weeks after delivery
	3D Ultrasound scan (2x vaginal)	2D Ultrasound scan (2x abdominal)		
Blood sampling	Blood sampling (1x)	Blood sampling	Blood sampling	Blood sampling
Anthropometric measurements	Anthropometric measurements (1x)			
Vaginal – rectal sampling	Vaginal – rectal sampling (1x)	Vaginal – rectal sampling (2x)	Vaginal – rectal sampling	Vaginal – rectal sampling
2 questionnaires diet and lifestyle	2 questionnaires diet/lifestyle (1x)	1 questionnaire (1x)	Placental biopsies	1 questionnaire
Inclusion in online platform 'SlimmerZwanger'			Umbilical cord blood sampling	
			Meconium sampling	

8.3.2 Extensive description of study procedures

Since this study is embedded in the Predict study, below is listed what measurements are additional for the PROMOTE study and which measurements are already part of the Predict study.

Clinical maternal outcomes (Predict): This information will be obtained via questionnaires included in the research module or by electronic medical records.

Maternal conditions and lifestyle (Predict): one/two extensive questionnaires about lifestyle, diet and environmental factors must be completed preconceptionally/in the first trimester, one general questionnaire in the second trimester at 24 weeks of gestation and during postpartum period. These questionnaires are fully digital, by using the GemsTracker program.

Anthropometric measurements (Predict): height, weight, chest circumference, waist circumference and hip circumference are measured during the intake.

Maternal blood samples (Predict/PROMOTE study): Blood sampling will take place at the outpatient clinic after the intake. The laboratory tests described down are in addition to the tests of the Predict study (leukocytes differential count and Homocysteine). For the PROMOTE study we will assess metabolic indicators such as glucose, insulin, cholesterol, HDL, LDL, lipids (standard methods), cytokines such as TNF α and IL6 (using Luminex), hsCRP (ELISA), measurements for oxidative stress such as total antioxidant capacity, protein oxidation products; carbonyl protein and advanced oxidation protein products (AOPP)) and parameters of the one-carbon metabolism including folate, homocysteine and vitamin B12. Part of the maternal and fetal (cord) blood samples will be prepared for staining in Rotterdam and frozen (in DSMO in liquid nitrogen) where after they are transported on dry ice to the UMCG in Groningen. Here they will be incubated with various panels of antibodies to study monocyte (subsets and activation status), granulocytes (activation status) and T cells (subsets and activation status). After antibody incubation, cells will be prepared for flow cytometry.

Neonatal cord blood/meconium sampling: the collection of neonatal cord blood will be performed at the labour ward, by the obstetrician on duty (as part of the Predict study). Measurements that will be assessed are extensively described at the

maternal blood samples. Meconium, as part of the PROMOTE study, will also be sampled at this time point in the study.

Placental tissue: Placental biopsies will be collected at labour ward and afterwards snap frozen. Presence of immune cells in decidua and oxidative stress will be characterized by immunohistochemistry. mRNA and protein will be isolated for gene (including epigenetics) or protein expression analysis (Dep of Reproduction and Development, prof J.Gribnau, Erasmus MC) for instance markers of the one-carbon metabolism, immunological markers, vascular markers, cytokines, markers of oxidative stress.

Vaginal/Faecal sampling/Microbial sequencing: To analyse the composition and differentiation of the maternal microbiome: maternal rectal and vaginal samples will be collected by the women during their study visits at the hospital (1st trimester, 2nd trimester and third trimester, during delivery and during postpartum checkup). The bacteriome profiles will be assessed by 16SrRNA gene amplication sequencing by the department of microbiology to analyse the composition of the maternal microbiome. Bacterial 16S rRNA gene sequences covering variable regions (V6–V8) will be PCR amplified from purified genomic DNA by using the standard primers. Sequences will be assigned to OTUs (operational taxonomic units).
Virome sampling will be performed during clinical visits, and will be stored and analysed according to standardized protocols by the department of Viroscience Erasmus MC (Prof. Dr. Koopmans).

8.3.3 Standard procedure of ultrasound scans

At 7, 9 and 11 weeks GA the gestational sac, embryo, and placenta are depicted in 3D ultrasound scans using a GE Voluson E8 or E10 Expert system and 4D View software (GE Medical Systems, Zipf, Austria). At 22 weeks and 30 weeks of GA standard biometry; Doppler measurements: arteria umbilicalis, arteria media cerebralis and arteria uterine will be performed.

In this study transvaginal scanning time (during first trimester) and transabdominal scanning time (second and third trimester) will be kept as short as possible. The duration of the scanning time per ultrasound will not exceed 30 minutes in the first trimester and 40 minutes in the second and third trimester.
All scans will be made with standard settings of the ultrasound machine: pulse repetition frequency of 0.6 kHZ, gain -2.0, quality “high’’, wall motion filter ‘’low’’.
The obtained 3D datasets are stored as Cartesian (rectangular) volumes.

Pulsed wave Doppler is a standardized additional modality of ultrasound imaging to quantify blood flow. Blood flow will be quantified as expressed by the resistance index (RI) as well as by the pulsatility index (PI). Hence, the use of pulsed wave Doppler signal during ultrasound enables us to perform a non-invasive measurement of the blood flow and subsequently to detect changes in flow. All the ultrasounds are part of the Predict study.

8.3.4 Sample collection

Serum, rectal and vaginal samples will be obtained during preconceptional, first trimester, second trimester, third trimester, during delivery and after postpartum period. All samples will be processed and stored at each setting according to standardized protocols.

8.4 Withdrawal of individual subjects

Subjects may decide to withdraw from the study at any time, for any reason if they wish to do so, without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal

Not applicable

8.5 Replacement of individual subjects after withdrawal

Not applicable

8.6 Follow-up of subjects withdrawn from treatment

Not applicable

8.7 Premature termination of the study

Not applicable

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

The interventions performed in this pilot study are blood collection, vaginal and rectal swab collection.

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to an intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

Not applicable.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable.

9.3 Annual safety report

Not applicable.

9.4 Follow-up of adverse events

Not applicable.

9.5 Data Safety Monitoring Board (DSMB) / Safety Committee

Not applicable.

10. STATISTICAL ANALYSIS

10.1 Primary study parameter(s)

Since this study is a pilot, including 60 obese and 60 non-obese women, exploratory power calculations are not performed at this stage. Furthermore, performing a power calculation would require prior estimates of all parameters in a model explaining the relation between the measurements and the outcomes, which is the goal of this study.

Descriptive statistics will be presented for maternal and lifestyle conditions and such as but not limited to; medication use, intoxications, infections, physical activity, working activities, blood pressure, nutrition, smoking, alcohol, and folic acid supplement use to give an overview of the data. We will use mean and standard deviation for variables that are approximately normally distributed, median and interquartile rand for variables that do not appear to have a normal distribution.

We will investigate the microbiome by using different analyses. The alpha-diversity (microbiome within a sample) will be described using the Shannon-index. We will use univariate analysis to describe and compare alpha-diversity per person and per analysis group (normal BMI and BMI >30) by chi-square test. These descriptions will be presented and compared at different time points (preconceptional, 1st trimester, 2nd trimester, 3rd trimester, postpartum).

The alpha diversity will further be analysed using a marginal model for repeated measurements (fitted using generalized least squares). BMI-group, time and their interaction will be included as covariates as well as the potential confounders ('parity, age, mode of conception, infection, and fetal gender'). Several common variance-covariance structures will be used and the one that achieves the best fit according to Akaikes information criterion will be used for further analyses. A likelihood ratio test will be used to test the hypothesis that the evolution of the alpha diversity differs between the BMI groups.

Beta diversity (variation of microbial communities between the samples) will be examined per time unit (possibly preconceptional, 1st trimester, 2nd trimester, 3rd trimester, postpartum), per person and between the 2 groups of persons (normal BMI and BMI > 30) at the different time units (1st trimester, 2nd trimester, 3rd trimester, postpartum). The possible changes in microbiome over time in the different BMI groups will be analysed with a confidence interval based on bootstrapping. Potential confounders (parity, age,

mode of conception, infection, and fetal gender) will be also corrected for. Missing data will be handled by multiple imputation. A p-value <0.05 will be considered statistically significant unless otherwise stated.

10.2 Secondary study parameter(s)

We will investigate the association between the composition of the microbiome and the maternal, fetal and pregnancy outcomes by using a multivariate linear or logistic regression model. A multivariable linear regression is used for the association between the microbiome (in alpha and beta diversity) and the continuous outcomes (birth weight, placenta weight, immune response and one-carbon metabolism (markers with continuous outcome). The multivariable logistic regression model is used for the association between the microbiome and the dichotomous outcome measures: (hypertension yes/no, pre-eclampsia yes/no, gestational diabetes yes/no).

The association between the composition of the microbiome (alpha and beta-diversity) at different timepoints (preconceptional, 1st trimester, 2nd trimester, 3rd trimester and postpartum) and fetal growth (at 1st, 2nd and 3rd trimester), will be analyzed by a linear mixed effect model (repeated measurements in the same person). We stratify for normal BMI and BMI>30 and also include BMI among the potential confounders (parity, age, mode of conception, infection and fetal sex).

Continuous, normally distributed variables will be presented as mean with standard deviation, and variables with a skewed distribution as median with the range. Categorical variables will be presented as count and proportions. The independent samples t-test and Mann-Whitney U test will be used for continuous data. Two-sided p-values less than 0.05 will be considered statistically significant.

10.3 Other study parameters

Not applicable.

10.4 Interim analysis

Not applicable.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (version October 2013) and in accordance with relevant national guidelines, regulations and Acts (e.g., for Erasmus MC the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and consent

For recruitment of high risk groups of participants and their (unborn) children we will use the infrastructure of the preconception, early pregnancy and reproductive life planning clinics of Erasmus MC. Participants can enter the study during the preconception period and/or at <13 weeks GA.

Women contemplating pregnancy or being pregnant and visiting the preconception and reproductive life planning clinics at Erasmus MC will be informed by their clinician and invited to participate in the cohort. Participation is fully voluntary. Participants will be informed about the voluntary nature of their participation by their clinician as well as by means of the information leaflet and informed consent form. Full comprehensive information will be given to all participants according to the regulations of the authorities that need to approve the clinical studies. Subjects that will be addressed are:

- research objectives
- the nature, extent and duration of the procedures
- details of the additional risk and burden caused by the research project and the protective measures to prevent or address the potential problems
- possible adverse events
- voluntary participation
- the opportunity to ask questions and receive understandable answers before making a decision
- who will benefit from participation
- the procedures that will be implemented in case of incidental findings
- how their data will be collected, protected during the project and either destroyed or reused at the end of the research
- being able to withdraw themselves and their data from the project at any time
- any potential commercial exploitation of the research

Informed consent:

The study will be performed according to written informed consent procedures and the study protocol approved by the METC Erasmus MC. The informed consent procedures ensure personal data protection and confidentiality. Data will be coded, i.e. information will not be directly traceable to an individual person.

Written information will be presented clearly, using short sentences and either avoiding or explaining all technical terms. Information sheets will be provided in the participants' own languages. Participants will be informed about the study according to their understanding and need to provide written consent to participate, only after a full explanation has been given and understood, an information leaflet offered and time allowed for consideration. In case of inclusion during pregnancy, additional signed consent regarding the use of the child's data is requested from both parents (i.e. the legal representatives of the child). The right for the participant (and/or the legal father) to refuse to participate without giving reasons will be respected, they are free to withdraw at any time from the protocol treatment without giving reasons and without prejudging further treatment.

11.3 Objection by minors or incapacitated subjects

Not applicable

11.4 Benefits and risks assessment, group relatedness

Information gained by ultrasound will provide doctors and parents with information. It is also hoped that the information gained from this study will be valuable to future parents to assess the influence of obesity on the pregnancy and outcomes.

Risks: Worldwide ultrasounds are routine antenatal care. Following specifications as set by ISUOG, the assumed risks with respect to ultrasound examinations and phlebotomy are very minimal.

For all participants the risks involve primarily the burden of participating in a study, which means additional hospital visits and additional assessments. All efforts will be taken to limit the number of visits and assessments and to harmonize the visits and assessments with usual clinical care. The risks of participation are considered to be minor and the potential benefit outweighs the risks. A detailed risk assessment will be included in the study protocol, informed consent documents and other study documents delivered to the ethic committees. From the above, it is clear that there are no obvious risks associated

with participation in the study and that participants included will benefit from receiving more clinical care.

11.5 Compensation for injury

The Erasmus MC has a liability insurance which is in accordance with article 7 of the WMO.

The Erasmus MC (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study. However, as participation in this study involves, at most, a negligible risk for injury the METC Erasmus MC imparted a waiver for this insurance.

11.6 Incentives

Study participants will receive 2D and 3D ultrasound examinations and pictures, if possible, as compensation for their participation.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Individually identifiable private information

Individually identifiable private information will only be accessible to clinicians involved in the clinical care of the participant, the research team, the study monitor, and IGJ (Inspectie Gezondheidszorg en Jeugd). Importantly, no contact details will be included in datasets that are obtained from each participant and no identifying details will leave the participating centre.

Protection of data

The Erasmus MC will provide data in a format using a common data model that will be defined upfront. Data will be de-identified (pseudo-anonymised) in order to guarantee the privacy of the participants, by assigning a study-ID. We will keep a (strictly confidential) mapping from their local patient ID to the study-ID.

Safety of assessments

The as-low-as-reasonable achievable (ALARA) principal has been recommended in statements of the Food and Drugs Administration (FDA), American Institute of Ultrasound in Medicine (AIUM), World Federation of Ultrasound in Medicine and Biology (WFUBM),

International Society of Ultrasound in Obstetrics and Gynaecology (ISUOG), European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) and Australian Society for Ultrasound in Medicine (ASUM) regarding medical use of ultrasound. 3D ultrasound imaging is not associated with higher risks than two-dimensional (2D) B-mode ultrasound, as 3D is a post-processing procedure using captured fields. Off-line evaluation of stored 3D images furthermore reduces the duration of exposure time in comparison to the use of real time 2D. At present Power Doppler ultrasound is not used routinely in clinical practice. When performing Doppler ultrasound in the first trimester of pregnancy, the displayed TI should be ≤ 1.0 and exposure time should be kept as short as possible (usually no longer than 5 -10 min) and should not exceed 60 min. The proposed study will comply with above mentioned recommendations.

Security of personal data

The study-consortium considers the safety and security of the personal and individual data obtained and stored in the study-database hosted by participant Erasmus MC to be most important. All data will be collected, stored, processed and disclosed in compliance with national regulations. Data transfers within the EU/EEA are not subject to specific requirements (i.e. specific authorizations or other restrictions) when complied with the general requirements of Directive 95/46/EC. All national laws and regulations (e.g. Code of conduct on health research and Personal Data Protection Act) on data protection will be respected.

Storage of data

All data entered by the participants will be stored in the database hosted by Erasmus MC. All imaging data will be stored and handled in the same way and according to the same rules and regulations as other patient related imaging data at Erasmus MC. It will be stored as files on a separate research storage platform. Access will be limited to authorized medical personnel. Should data transfer to other parties within the study-consortium be necessary for testing or validation purposes, we will code the datasets and remove all personal data before the transfer.

Privacy of data

The data will always be handled confidentially and coded using a random identification code. The key to this code will be kept separately from the dataset at all times. If datasets are to be transferred to other locations this will only be performed after stripping them of all identifiers, leaving only the subject identification code. The key will remain on site with the local research team.

Study-database and disclosure of data

With respect to the existing clinical observational datasets, all research data will be retained and stored in the study-database at the research institutions where data were primarily collected. Ownership of the data remains with the institutions that collected the data. Any data transferred from one centre to another will be stripped from all identifying variables, i.e. completely anonymized. If for verification purposes it is not possible to completely anonymize, files will be coded using a random identifier number. The key to this identifier number will at all times remain with the local research team at the institution where the data was collected. Any data transferred or combined by one of the consortium partners will be stored at the secured server of the partner's institute during the running of the project. It will remain there for at least 15 years after completion of the project.
Due to the sensitive nature of the participant's data, datasets will never be made publicly available. Data will only be stored at secured servers of the participating research centres. All researchers who are not seated in the primary consortium and who are given access to the confidential information that has been pseudo-anonymised must provide a signed statement that they will maintain confidentiality.
The Principal Investigator agrees to archive the study documents for 15 years from the study end.

Data management

Any data storage and handling process will ensure data protection and confidentiality. The data entered in the study-database will be coded using a study-ID. Results from ultrasound examinations will be supplied in a file. Standardized electronic files will be used that supply output with a unique personal code without information that is directly traceable to the person. For extraction of data for research purposes by external parties, the study-ID will be removed. In the absence of this ID, researchers cannot identify the individual subjects. All data will be stored on hospital computer systems. All project data will be back-upped automatically via the O:disk and copies will be kept in two separate locations.

Disclosure of data

The personal identification data will not be made available to persons outside the local research team, including the other researchers in the study-consortium. Results of research will be published without any reference to individual participants of the study.

Accountability

The study-consortium is responsible for the data collection, secure storage and disclosure of data. Any misconduct can be directed to the coordinating investigator of the study-consortium.

12.2 Monitoring and Quality Assurance

The risk of this study is qualified based on the guideline by the NFU (Dutch Federation of University Medical Centers) about quality insurance in human research ("Kwaliteitsborging van mensgebonden onderzoek"). The NFU guideline states that the extent of risk has to be estimated by considering the additional risk of an intervention compared to standard treatment. The risk of this study is qualified as 'Negligible'.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last participant's last visit.
The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

Project results will be disseminated to healthcare professionals and to scientific and industrial peers through events, professional organisations and publications in peer-reviewed journals as well as presentations at scientific congresses.

The project will be communicated to the general public via a press release at the start of the project and by using the internal and external communication means of the partners, such as institutional websites, magazines and the yearly news letter from the Predict study. All data will be available for verification and re-use for at least 15 years. Full permission to access the data will be granted only to the principal investigator at the location where data are stored. In case of an additional research question, this research can only be done with permission of all members of the Leadership Team. Before access is granted to any person outside of the consortium, a signed statement ensuring confidentiality must be obtained.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action

Not applicable.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

Not applicable.

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

Not applicable.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Not applicable.

e. Analysis of potential effect

Not applicable.

f. Pharmacokinetic considerations

Not applicable.

g. Study population

Not applicable.

h. Interaction with other products

Not applicable.

i. Predictability of effect

Not applicable.

j. Can effects be managed?

Not applicable.

13.2 Synthesis

Not applicable.

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