FETAL BRAIN CARE

Therapies for brain neurodevelopment in fetal growth restriction

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Date: December 2022

Setting: BCNatal | Fetal Medicine Research Center (Hospital Clínic and Hospital Sant Joan de Déu), Barcelona, Spain

Research line: Fetal brain development and fetal therapy

NCT: 05038462

ADMINISTRATIVE INFORMATION

This document provides information about an understanding of the background, rationale, objectives, and procedures for entering participants into the study, study population, interventions, methods, statistical analyses, ethical considerations, and administration of the study. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary. The study will be conducted in compliance with the approved protocol, the Declaration of Helsinki (2008), and the principles of Good Clinical Practice (GCP).

Structures study summary

Public title	Fetal Brain Care
Scientific title	Fetal Brain Care: Therapies for brain neurodevelopment in fetal growth restriction (FGR)
Contact for public queries	FETALBRAINCARE@clinic.cat
Contact for scientific queries	Chief investigators: Elisenda Eixarch, Míriam Illa, Eduard Gratacós Principal investigator: Elena Monterde BCNatal – Fetal Medicine Research Center (Hospital Clínic and Hospital Sant Joan de Déu), Barcelona, Spain
Setting of recruitment	BCNatal - Hospital Clínic and Hospital San Joan de Déu, Barcelona, Spain Hospital General de l'Hospitalet, Barcelona, Spain Hospital Germans Trias i Pujol, Barcelona, Spain Hospital de la Santa Creu i Sant Pau, Barcelona, Spain Hospital del Mar, Barcelona, Spain Hospital Dexeus-Quirón, Barcelona, Spain
Problem studies	Neurodevelopment in FGR
Participants	Pregnant women with FGR
Study type	A multicenter, randomized, double-blind clinical trial
Interventions	Maternal administration of 1000 mg of Lactoferrin + 1000 mg of DHA every day orally or placebo
Sample size	304 participants: 152 for each arm of randomization (Lactoferrin + DHA, placebo)
Inclusion and exclusion criteria	Inclusion: Singleton pregnancies Non-malformed fetus Pregnancies with FGR 24-32.6 weeks of gestation Exclusion: Chromosomal or structural abnormalities Critical Doppler

	Maternal mental or psychiatric disorders Maternal allergy to cow's milk protein
Data of first enrolment	January 2023
Study aims	To evaluate the impact of maternal supplementation with DHA and Lactoferrin on neurodevelopment in pregnancies with FGR, as assessed by fetal brain MRI and postnatal neurodevelopmental tests. To evaluate the impact of maternal supplementation with DHA and Lactoferrin on fetal growth assessed by prenatal ultrasound in FGR. To evaluate the impact of maternal supplementation with DHA and Lactoferrin on perinatal morbidity (neonatal acidosis, intraventricular hemorrhage III/IV, necrotizing enterocolitis, periventricular leukomalacia, sepsis, bronchopulmonary dysplasia) and mortality in FGR.

Roles and responsibilities

Name	Affiliation	Role
Elisenda Eixarch (EE)	BCNatal	Co-Chief investigator, Coordinator of Fetal Neurology Unit, specialist in Obstetrics and Fetal Medicine. General coordination and supervision of the project and analysis, interpretation and dissemination of results.
Míriam Illa (MI)	BCNatal	Co-Chief Investigator, Member of Fetal Neurology Unit, specialist in Obstetrics and Fetal Medicine. General coordination of the project, data collection supervision, neurosonography evaluation, supervision of fetal MRI acquisition and analysis, supervision of postnatal follow-up, and analysis, interpretation and dissemination of results.
Eduard Gratacós (EG)	BCNatal	Co-Chief investigator, Director of Department of Obstetrics, Gynecology and Neonatology of Hospital Clinic Barcelona, Director of BCNatal Center. General coordination and supervision of the project and analysis, interpretation and dissemination of results.
Elena Monterde (EM)	BCNatal	Principal investigator, specialist in Obstetrics and Fetal Medicine at Hospital Clínic. Patient recruitment, data collection, neurosonography evaluation, MRI analysis, supervision of postnatal follow-up, and analysis, interpretation and dissemination of results.
Francesc Figueras (FF)	BCNatal	Head of Maternal-Fetal Medicine Department at Hospital Clínic, specialist in Obstetrics and Fetal Medicine. Co-supervisor of patient recruitment at BCNatal, statistical support.
Mª Dolores Gómez Roig (DG)	BCNatal	Head of Gynecology and Obstetrics Department at HSJD, specialist in Obstetrics and Fetal Medicine. Co-supervisor of patient recruitment at BCNatal, statistical support.
Eva Meler (EMB)	BCNatal	Member of Intrauterine Growth Restriction Unit at Hospital Clínic, specialist in Obstetrics and Fetal Medicine. Co-supervision of fetal recruitment, clinical follow-up, and ultrasound evaluation.
Anna Peguero (AP)	BCNatal	Member of Intrauterine Growth Restriction Unit at Hospital Clínic, specialist in Obstetrics and Fetal Medicine.

		Co-supervision of fetal recruitment, clinical follow-up, and ultrasound evaluation.
Edurne Mazarico (EMG)	BCNatal	Member of Intrauterine Growth Restriction Unit at Hospital Clínic HSDJ, specialist in Obstetrics and Fetal Medicine. Co-supervision of fetal recruitment, clinical follow-up, and ultrasound evaluation.
Mónica Rebollo (MR)	BCNatal	Neuroradiologist at HSJD. Responsible of the acquisition, processing and analysis of fetal brain MR.
Marta Gómez-Chiari (MG)	BCNatal	Neuroradiologist at HSJD. Responsible of the acquisition, processing and analysis of fetal brain MR.
Carme Fons (CF)	HSJD	Head of Neurology Unit at HSJD, specialist in Paeditrician Neurology. Responsible of the neoantal follow-up and functional assessment.
Susana Fernandez (SF)	Hospital General Hospitalet	Coordination and patient recruitment in Hospital General de l'Hospitalet, data collection, clinical follow-up, and ultrasound evaluation.
Carmina Comas (CC)	Hospital Can Ruti	Coordination and patient recruitment in Hospital Germans Trias i Pujol, clinical follow-up, and ultrasound evaluation.
Beatriz Lorente (BL)	Hospital Can Ruti	Patient recruitment in Hospital Germans Trias i Pujol, data collection, clinical follow-up, and ultrasound evaluation.
Irene Ribera (IR)	Hospital Sant Pau	Coordination and patient recruitment in Hospital de la Santa Creu i Sant Pau, clinical follow-up, and ultrasound evaluation.
Antonio Fernandez (AF)	Hospital Sant Pau	Patient recruitment in Hospital de la Santa Creu i Sant Pau, data collection, clinical follow-up, and ultrasound evaluation.
Jose Luis Hernandez (JLH)	Hospital del Mar	Coordination and patient recruitment in Hospital del Mar, data collection, clinical follow-up, and ultrasound evaluation.
Gerard Albagies (GA)	Hospital Dexeus-Quirón	Coordination and patient recruitment in Hospital Dexeus-Quirón, data collection, clinical follow-up, and ultrasound evaluation.
Erika Muñoz (EMZ)	BCNatal	Lab technician. Coordinator of sample collection and storage, sample analysis.
Nuria Sebastian-Galles (NS)	Univesitat Pompeu Fabra Barcelona	Coordination of the neurodevelopmental assessment at Speech Acquisition and Perception Lab.
Chiara Santolin (CS)	Univesitat Pompeu Fabra Barcelona	Performance of the neurodevelopmental assessment at Speech Acquisition and Perception Lab.

ABBREVIATIONS

AC	Abdominal circumference
ADHD	Attention deficit hyperactivity disorders
BPD	Biparietal diameter
CPR	Cerebroplacental ratio
DCS	Diffuse correlations spectroscopy
DHA	Docosahexaenoic acid

DV	Ductus venosus
ECG	Electroencephalography
ET	Ejection time
FEW	Fetal estimated weight
FGR	Fetal Growth Restriction
FL	Femur length
GCP	Good Clinical Practice
HC	Head circumference
HSJD	Hospital Sant Joan de Déu
ICT	Isovolumetric contraction time
IRT	Isovolumetric relaxation time
MCA	Middle cerebral artery
MPI	Myocardial performance index
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NST	Non-stress test
PI	Pulsatility index
PIm	Mean pulsatility index
PSQI	Pittsburg Sleep Quality Index
PSS	Perceived Stress Scle
RCOG	Royal College of Obstetrics and Gynecologists
RCT	Randomized Controlled Trial
RF	Radio frequency
TAPSE	Tricuspid Annular Plane Systolic Excursion
UA	Umbilical artery
UtA	Uterine artery
WHO	World Health Organization

INTRODUCTION

Two-thirds of the neurodevelopmental disorders are estimated to be related with an insult occurring during the prenatal period and fetal growth restriction (FGR), that occurs in 7-10% of pregnancies, can account for up to one-quarter of cases of special educational needs. However, there is currently no treatment to prevent or ameliorate the effects of FGR on neurodevelopment. Promising experimental results have shown that maternal supplementation with Lactoferrin and docosahexaenoic acid (DHA) has strong neuroprotective effects in fetuses with FGR. However, no clinical trials have evaluated their effects in humans. We propose a randomized double-blind placebo-controlled trial to assess whether Lactoferrin and DHA can improve neurodevelopment in FGR fetuses and infants.

Prenatal origin of altered neurodevelopment

Neurodevelopmental disorders emerge early in life, and two-thirds are estimated to be related with an insult occurring during perinatal period. Fetal growth restriction (FGR) is a prevalent condition that affects 7-10% of all pregnancies in developed countries, being associated with short- and long-term neurodevelopmental problems, including motor and cognitive delay (1). For instance, a recent cohort study showed that one-quarter of schoolchildren with special educational needs due to sensory, motor and intellectual disabilities were related to FGR combined with prematurity (2). FGR is also associated with a higher risk of autism spectrum (3) and attention deficit hyperactivity disorders (ADHD) (4). Moreover, brain macro- and microstructural, as well as metabolic and connectivity alterations underlying neurodevelopmental dysfunctions have been characterized in subjects with FGR at different stages of development, starting in utero (5) and persisting postnatally during early infancy (6) and adolescence (7). Despite the prevalence and impact of this condition in developed societies, there are no effective prenatal therapies to either improve placental function, or to prevent its deleterious effects on neurodevelopment.

Therapeutic strategies in perinatal period

Several evidences suggest that early postnatal life interventions, such as breastfeeding, may improve neurodevelopmental outcomes in FGR (8). Furthermore, experimental data demonstrates that postnatal environmental enrichment can reverse FGR effects on brain function and structure, by improving memory, reducing anxiety, as well as refining the organization of brain connections and increasing dendritic spines (9). However, all these strategies are applied after birth, when the adverse effects of FGR on brain development have already been established. Prenatal life represents the optimal window of opportunity to reduce the long-term brain consequences of FGR. Brain development is a complex process that starts early in utero and is prolonged after birth, conferring a high sensitivity to any circumstance, either positive or negative. This period, which includes the prenatal period, is called "critical window of opportunity" and any strategy applied during this episode could have a more pronounced effect on brain function and structure, thus reverting more effectively the adverse consequences of prenatal insults, such as FGR (10).

Prenatal therapy should be non-invasive, ensure placental crossing and have an impact on the pathophysiological mechanisms. Among the potential strategies aimed at protecting neurodevelopment under FGR, DHA and Lactoferrin emerge as good candidates. DHA is a fatty acid that is transferred across the placenta to reach fetal circulation and exerts a central role in the central nervous system development (11). Poor maternal DHA intake has been related with delayed brain and retinal development (12) and its administration after hypoxic-ischemic injury

improved long-term anxiety and memory in rats (13). On the other hand, Lactoferrin is found in human milk and crosses the blood brain barrier once it has been absorbed in the gastrointestinal tract. Lactoferrin modulates a wide range of neuronal processes and various mechanisms have been proposed to explain its neuroprotective effects (14). Animal studies strongly suggest that maternal Lactoferrin supplementation has a fetal brain protective role in placental insufficiency (15) and preterm hypoxic-ischemic injury (16).

Evidence supporting the neuroprotective effect of DHA

Previous studies have suggested a beneficial effect of DHA on brain function and structure. At experimental level, the administration of DHA in a hypoxic-ischemic rat model (13) was related with a long-term improvement in anxiety and memory. In addition, imaging studies in primate linked DHA administration with cortical improvement in aging and in neuropsychiatric diseases, including schizophrenia (17) and ADHD (18). Moreover, enhancing the nutrient supply with DHA to FGR infants born prematurely improved white matter maturation measured by DTI at term-equivalent age (19). Administration of DHA has also demonstrated to improve dendritic spine density (20) and morphological alterations (20,21) in adulthood. The effect of maternal supplements containing DHA has also been evaluated in several clinical trials. A recent review summarizing the results of these studies concluded that there is contradictory evidence on the potential beneficial effect on neurodevelopment during childhood (22,23). Accordingly, whereas administration of the DHA did not improve neurodevelopment in general population, it might be beneficial in high-risk population.

Evidence supporting the neuroprotective effect of Lactoferrin

Although no clinical studies have evaluated the role of Lactoferrin on neurodevelopment, experimental research has proposed Lactoferrin for neuroprotection during early stages of life. Maternal administration of Lactoferrin in the perinatal period has been associated with brain metabolism restoration in both placental insufficiency (15) and preterm hypoxic-ischemic (16) rat models. Lactoferrin administration during breastfeeding in a preterm rat model also resulted in an amelioration of the microstructural brain damage detected by diffusion weighted imaging studies in specific brain areas, such as the striatum, corpus callosum and external capsule in the neonatal period (16)(24). Moreover, an increased rate of learning and long-term memory was observed with Lactoferrin supplementation in piglets (25). Additionally, both DHA and Lactoferrin have been demonstrated to have a neuroprotective role in several neurological disorders, including Parkinson's disease, schizophrenia and ADHD (18,26,27).

Altogether, despite the existence of indirect evidence, direct proof of the protective effects of DHA or Lactoferrin administration prenatally in FGR remained elusive.

Experimental data supporting the potential benefit of DHA and Lactoferrin in FGR

The present proposal is the result of years of investigation on brain development in FGR conducted by our research group. An important contribution was the demonstration that FGR is strongly associated with altered neurodevelopment, even in its milder clinical forms. We have previously described how this effect was mediated by increased brain perfusion, and we introduced the use of fetal brain Doppler for the identification of high risk cases (28–32). In addition, the group pioneered the use of advanced MRI techniques to demonstrate macro- and microstructural, as well as metabolic, connectivity and functional brain changes not only in the postnatal stage (6,33–38), but also in prenatal life (5,39–43). These evidences of abnormal brain

development in FGR constituted the starting point to focus our strategy on preventing prenatal brain damage.

In parallel with clinical studies, we developed and validated an animal model of FGR in pregnant rabbits that reproduces clinical features of FGR including high perinatal mortality, reduced birth weight, hemodynamic changes and structural/functional changes of the brain (30,44–49). Once the model was validated, we tested our hypothesis regarding prenatal administration of specific therapy to revert, partially or totally, the deleterious effects of FGR on the developing brain. We conducted a serial of experiments in our animal model of FGR, obtaining promising results with DHA and Lactoferrin. In our model, maternal administration of DHA or Lactoferrin during pregnancy was associated with neurodevelopmental improvement at the neonatal and at 70 days of life (equivalent to adolescence in humans), at both structural and functional levels (49). At neonatal period, maternal administration of DHA and Lactoferrin improved neuronal arboritzation and reversed oligodendrocytes dysmaturation observed in FGR animals, especially after DHA administration. At long-term, period, maternal administration of DHA and Lactoferrin improved brain connectivity at macro- and micro- levels, by increasing global efficiency of structural brain networks and improving hippocampal perineuronal networks and dendritic spines. Functional improvements could also be observed by means of reduced anxiety and better memory and learning competences. This study provided for the first-time evidence that fetal neurodevelopment improved after maternal supplementation with DHA or Lactoferrin in FGR.

National or international groups working in the same specific or related lines

Regarding the impact of FGR in brain development, our research group pioneered in demonstrating its effects in both brain structure and function, being one of the most productive research groups in this field nowadays. Other groups working in the same field include the Child Development Disorders in the University of Geneva directed by Petra Huppi and the Center for the developing Brain in Kings College directed by David Edwards. They mainly focused on the development of imaging biomarkers based on MRI and are currently working in different strategies to prevent severe brain damage, particularly in preterm deliveries.

In summary, the present research protocol aims at testing the positive results observed in animal models in a clinical study with pregnant women. If successful, this study will provide the first supporting evidence of an effective strategy to reduce the neurodevelopmental impact of FGR, moving a step forward that will open the door to therapies with the potential to improve the neurodevelopmental health of thousands of children yearly. Furthermore, this evidence could open new research pathways and the opportunity to apply similar strategies in other perinatal conditions associated with poor neurodevelopment, including toxic exposure, prematurity and cardiac defects.

STUDY AIMS

Hypothesis

Main hypothesis:

1. A prenatal intervention based on maternal supplementation with DHA and Lactoferrin improves neurodevelopment in FGR.

Secondary hypothesis:

1. A prenatal intervention based on maternal supplementation with DHA and Lactoferrin in FGR improves fetal growth.
2. A prenatal intervention based on maternal supplementation with DHA and Lactoferrin in FGR improves perinatal morbidity and mortality.

Objectives

Main objective:

1. To evaluate the impact of maternal supplementation with DHA and Lactoferrin on neurodevelopment in pregnancies with FGR, as assessed by fetal brain MRI and postnatal neurodevelopmental tests.

Secondary objectives:

1. To evaluate the impact of maternal supplementation with DHA and Lactoferrin on fetal growth assessed by prenatal ultrasound in FGR.
2. To evaluate the impact of maternal supplementation with DHA and Lactoferrin on perinatal morbidity (neonatal acidosis, intraventricular hemorrhage III/IV, necrotizing enterocolitis, periventricular leukomalacia, sepsis, bronchopulmonary dysplasia) and mortality in FGR.

METHODS

Study design

A multicenter, randomized, double-blind clinical trial on 304 pregnant women with FGR at 24-32 weeks of gestational age randomized to: (1) Maternal administration of 1000 mg of Lactoferrin + 1000 mg of DHA every day orally or (2) placebo.

The study design of the RCT adheres to standard criteria for randomized trials, and it is registered in the Clinical Trials Gov (https://clinicaltrials.gov/) with the following NCT: 05038462.

Study population

Singleton pregnancies being diagnosed of FGR from 24 to 32.6 weeks of gestation. FGR will be defined either by (1) an estimated fetal weight (EFW) below the 3rd centile or (2) EFW< 3th centile or umbilical artery >95th centile or uterine artery mean pulsatility index > 95th centile.

After the recruitment, women will be randomized to two equally sized groups:

1. Lactoferrin + DHA
2. Placebo

Personnel who manage the patients in the clinical setting and also the patients will be blinded for each group of randomization the patient is belonged to.

Inclusion criteria:

- Singleton pregnancies
- Non-malformed fetus
- Pregnancies with FGR
- 24-32.6 weeks of gestation

Exclusion criteria:

- Chromosomal or structural abnormalities diagnosed during pregnancy or in the neonatal period.
- Critical Doppler study suggesting the need for delivery within the next 14 days at the time of diagnosis: reverse end-diastolic velocity in the umbilical artery or ductus venosus pulsatility >95th centile.
- Maternal mental or psychiatric disorders.
- Maternal allergy to cow's milk protein.

Study setting

The study will be coordinated by BCNatal, resulting from the integration of Hospital Clinic and Hospital Sant Joan de Déu in Barcelona (Spain), which is one of the most important university centers of maternal-fetal and neonatal medicine in Europe and a world-wide reference in this field. Both hospitals, with more than 7,000 deliveries per year, are university hospitals and tertiary referral institutions for high-risk pregnancies. They are part of BCNatal Fetal Medicine Research center, a research center in fetal and perinatal medicine, recognized as one of the world's best research teams in the field that has as a main objective to demonstrate how prenatal life has an impact on childhood and adult life. The center counts with a multidisciplinary

team of more than 70 members including doctors, engineers, biologists, nurses, psychologists as well as an own management structure.

Five external recruitment centers (Hospital Sant Pau, Hospital General Hospitalet, Hospital del Mar, Hospital Germans Trias i Pujol, Hospital Dexeus-Quirón), will take care of standard clinical follow-up, delivery and biological samples collection.

Neurodevelopmental assessment will be carried out in collaboration with the Speech Acquisition and Perception Groups of the Universitat Pompeu Fabra Barcelona under the supervision of Nuria Sebatian-Galles.

Sample size estimation

A final total sample size of 304 cases of FGR has been estimated based on the main outcome measures of the study: improvement in 5 units in the treated group in comparison to non-treated one with a SD of 13 units (as reported in Shavchev S, Ultrasound Obstet Gynecol 2013; 42: 201–206) with a 80% of statistical power with a risk of type 1 error of 5%. We also have estimated a 30% loss to follow-up rate.

Recruitment

For the recruitment it is important to identify those patients at a higher risk to develop FGR. The criteria to identify those patients are based on the criteria established by the RCOG (50). We will also identify those patients with a FEW <p10 or PImAU >p95 on the second trimester ultrasound. Patients presenting any of these criteria, serial ultrasound assessments would be scheduled, at 24, 28 and 32 weeks of gestation. We will provide those patients with an information sheet of our study in order to contact us in case they want to participate if FGR is diagnosed during pregnancy. We will also identify the FGR in the specific unit of the different centers that participate in the current study.

Enrolment visit

Pregnancies with a diagnose of FGR between 24-32.6 weeks of gestation are considered eligible for this clinical trial and the same day of the first evaluation in the specific unit of each center they will be identified by one of the investigators involved in the recruitment.

The visit serves to identify inclusion/exclusion criteria in a more comprehensive manner and to address all eligible patients about the purpose of the study and the voluntary nature of the participation. If the patient agrees to participate, she will be referred to BCNatal for the enrolment visit.

This enrolment visit includes:

1. A face-to-face interview in order to identify any exclusion criteria. If the candidate meets all the requirements, an informed consent form will be given to her to be signed after a detailed explanation of all study's procedures. The informed consent comprised two parts, one for study participation (Appendix 1) and another for biochemical analyses and DNA collection for genetic analyses.
2. Socio-demographic data, clinical history, anthropometric measures and blood samples for the assessment of basal maternal levels of DHA and Lactoferrin, angiogenic factors and cytokines will be taken.
3. Several questionnaires will be given and explained to the patient for a self-report to be completed at home.

4. Randomization.
5. Medication delivery.
6. The patient will be informed about the contact email of the study in order to address specific questions or problems related to the study.
7. Next appointments will be given to the patient.

Randomization

After obtaining the informed consent, the patient will be randomized in one of the two arms of this trial. Patient randomization will be done through a dedicated website for the study. Randomization sequences will be generated to assure balanced distribution within study arms (cluster randomitzation 12:12). The randomization list will be unknown to investigators and recruitment centers. Patients and clinicians will be blinded to the nature of study drug: supplements or placebo (double-blind).

Intervention

The idea behind the intervention is focused in maternal supplementation: the neuroprotective effects of maternal supplementation with Lactoferrin and DHA could prevent or ameliorate the effects of FGR on neurodevelopment. That intervention is based in robust scientific evidence as explain above.

The intervention consists in the maternal administration of 1000 mg of Lactoferrin + 1000 mg of DHA every day orally. The intervention will start at the time of inclusion and will finish at delivery.

Placebo with the same appearance, weight and taste will be also administrated daily until the end of pregnancy.

Participant timeline

Promotion of adherence

Efforts to promote adherence begins at the earliest stages of the study. During the first visit at enrollment, participants are repeatedly provided with information about key features of the study. Specific actions will be planned in order to minimize loss to follow-up, which is one of the potential limitations of this study. During the prenatal period, such cases are expected to be few since diagnosis of FGR implies a close clinical monitoring by fetal ultrasound weekly. A personal work-note will be given to each participant in order to register the compliance of the intervention to ensure a high adherence. However, cases lost could increase after delivery. Aiming to reduce these cases, reminders will be sent by phone and email to patients.

Assessment of compliance

The compliance of the intervention is essential and people involved will reinforce this concept. For analysis purposes, the intervention will be considered complete if treatment with at least 90% adherence is maintained at least for 4 completed weeks. Since the intervention is usually well tolerated and no major side effects have been described, we do not expect to stop or modify the intervention secondary to side-effects or harms. During the intervention period, clinicians will encourage and ask directly about compliance and the personal work-note will be revised in every weekly follow-up visit. At the end of participation in the study, participants will be asked to return the excess of medication in order to estimate the adherence. Finally, biomarkers for

treatment compliance will be assessed in a random subgroup of 100 women by a dosage quantification of both Lactoferrin and DHA in maternal and cord blood samples.

Desertion from the study

Data from patients enrolled in the trial who wants to desert it for any reason will be registered.

Measurements

All participants will receive an intervention divided in several phases:

1. Inclusion and randomization at 24-32.6 weeks of gestation:
    - Signature of informed consent form
    - Register of socio-demographic data
    - Randomization (randomization arms 12:12)
2. Administration of treatment/placebo according to randomization group:
    - Maternal administration of 1000 mg of Lactoferrin + 1000 mg of DHA every day orally
    - Placebo with the same appearance, weight and taste will be also administrated daily
3. Nutritional and lifestyle interviews and maternal blood sampling at inclusion:
    - Nutritional interview: adherence to the Mediterranean diet within a 17-items scale
    - Lifestyle interview: Perceived Stress Scale, Pittsburgh Sleep Quality Index and WHO-5 Well-being Index
    - Maternal blood sampling in a random subgroup of 100 women: angiogenic factors, cytokines and DHA/Lactoferrin levels
4. Prenatal follow-up:
    - Fetal growth and feto-placental Doppler every 7-14 days according to the standard clinical protocol
    - Neurosonography at the moment of enrolment and at 32 and 36 weeks of gestation
    - Fetal brain MRI at 34 weeks of gestation
    - Speech discrimination task at 36 weeks of gestation
    - Echocardiography following the standard clinical protocol at 32 weeks of gestation
    - Nutritional and lifestyle interviews at 36 weeks of gestation
    - Computerized non-stress test (NST) at inclusion, 28 and 36 weeks of gestation in those patients with Doppler alteration following the standard clinical protocol
5. Collection of perinatal data at delivery
6. Placental pathology
7. Maternal blood sampling at delivery in a random subgroup of 100 women: angiogenic factors, cytokines and DHA/Lactoferrin levels
8. Fetal cord blood sampling at delivery in a random subgroup of 100 neonates: DHA/Lactoferrin levels
9. Postnatal follow-up:
    - Blood pressure and heart rate at 6 and 24-month of corrected postnatal age
    - IM thickness and head circumference at delivery, 6 and 24-month of corrected postnatal age
    - Neurodevelopmental assessment:
        - Speech discrimination task at 4-month of corrected postnatal age
        - Attention switching task at 12-month of corrected postnatal age
        - Vocabulary assessment at 18, 24 and 36-month of corrected postnatal age
        - Logical reasoning task at 19-month of corrected postnatal age
        - Bayley III test at 6 and 24-month of corrected postnatal age
        - Early Childhood Behavior Questionnaire for temperament at 24-month of corrected postnatal age

Maternal nutritional and lifestyle assessment

At the moment of recruitment, participants will receive a pack containing one of each of the questionnaires below detailed. All questionnaires are self-reported.

- Diet quality: the adherence to the Mediterranean diet within a 17-items scale will be registered for each.
- Anxiety and stress: The Perceived Stress Scale (PSS) is one of the best currently available instruments to evaluate the presence of anxiety and stress during pregnancy (51,52). The WHO-5 Well-being Index will be also registered.
- Sleep quality: The Pittsburgh Sleep Quality Index (PSQI) is based on eighteen self-reported questions about the person own sleep quality. The scale evaluates seven rated components, including, sleep subjective, quality, duration, disturbances, and latency, habitual sleep efficiency, use of sleeping medication, as well as daytime function. The score from each category is added to achieve a global score that range from 0 – 21. A cutoff score of 5 or above is indicative of a sleep disturbance. This scale has been recently validated in the obstetric population (53), in which an abnormal result was associated with an increased risk of preterm birth (54).

At 36 weeks of gestation, participants will receive again the pack containing one of each of the nutritional and lifestyle questionnaires.

Prenatal follow-up

Fetal growth assessment

Fetal biometry: includes evaluation of fetal growth using the Hadlock formula (55) based in a composite sonographic measurement of fetal head (biparietal diameter, BPD; head circumference, HC), abdominal circumference (AC) and femur length (FL). Fetal measurements will be performed following previously published techniques.

Feto-placental Doppler assessment

Umbilical artery (UA) pulsatility index (PI) will be performed from a free-floating cord loop. Normal UA will be considered as a PI below the 95th percentile (56).

The middle cerebral artery (MCA) PI will be obtained in a transversal view of the fetal head, at the level of its origin from the circle of Willis (57–59). Three consecutive high-quality images with no artefacts will be recorded using previously reported parameters. Normal MCA will be considered as a PI over the 5th percentile.

The cerebroplacental ratio (CPR) will be calculated as a ratio of MCA PI to UA PI (60–62). Normal CPR will be considered as a PI over the 5th percentile. The MCA PI and CPR values below the 5th percentile will be considered indicative of cerebral blood flow redistribution (60–62).

The ductus venosus (DV) can be visualized either in a mid-sagittal longitudinal plane of the fetal trunk or in an oblique transverse plane through the upper abdomen. The sample volume will be positioned at its origin from de umbilical vein. The measurement has to be obtained in the absence of fetal breathing movements. Normal DV will be considered as a PI below the 95th percentile (63).

To examine the uterine artery (UtA), the probe has to be placed on the lower quadrant of the abdomen, angled medially and using the color Doppler to identify the UtA at the apparent crossover with the external iliac artery. Measurement will be taken approximately 1cm distal to

the crossover point. The PI of the left and right arteries will be measured and the mean PI will be calculated. Normal mean UtA will be considered as a PI below the 95th percentile (64).

Prenatal Doppler ultrasound examinations will be performed in the absence of fetal movements. Pulse Doppler parameters will be performed automatically from three or more consecutive waveforms, with the angle of insonation as close to zero as possible.

Fetal neurosonographic assessment

Fetal neurosonography will be performed at enrolment and at 32 and 36 weeks of gestation by an expert examiner at BCNatal using a two-dimensional transabdominal and transvaginal approach. Neurosonography will follow guidelines from the International Society on Ultrasound in Obstetrics and Gynecology (65), as well as guidelines for fetal brain assessment (66). Cortical development evaluation will be performed according *Pistorius et al*. (67), allowing quantification of brain sulcation in normal fetuses by ultrasound.

Fetal neurosonographic assessment will include the following parameters:

1. Axial planes:
    - Transventricular plane: lateral ventricles width, parieto-occipital fissure depth and grading, central sulcus grading.
    - Transthalamic plane: cephalic circumference, Sylvian fissure depth and grading, superior temporal fissure grading.
    - Transcerebellar place: transverse cerebellar diameter, antero-posterior diameter of cisterna magna.
2. Sagittal planes:
    - Midsagittal plane: corpus callosum (length, total area and regional areas(68)), craneo-caudal diameter of vermis.
    - Parasagittal plane: central sulcus grading.
3. Coronal planes:
    - Transcaudate plane: craneo-caudal diameter of the anterior horns, cingulate fissure depth and grading.
    - Transcerebellar plane: calcarine fissure depth and grading.

Neurosonographic images will be analyzed offline by experts blinded to group randomization, including grading and measurement of sulcal and fissure depths, together with delineations of the corpus callosum to obtain the total and regional areas.

Fetal Magnetic Resonance Imaging

Fetal brain MRI will be performed at 34 weeks of gestation in a 3 Tesla scan at BCNatal-Hospital Clínic, using a body array radio-frequency (RF) coil. Several sequences for the evaluation of fetal brain (micro-) structures and metabolic profile by MRS will be acquired. First, single-shot fast spin-echo T2-weighted sequences (TR 2010 ms; TE, 137 ms; slice thickness 3.5mm; no inter-slice gap acquisition) will be acquired in the three orthogonal planes, oriented along the axis of the fetal brainstem, obtaining 4-loops of transverse, 2-loops of coronal and 2-loops of sagittal single shoot slices. MRS data will be acquired from the frontal lobe based on T2-w reference images and with Point Resolved Spectroscopy (PRESS) localization, as previously reported (43). The final MRI acquisition protocol will be adjusted so that it does not exceed 30-45 minutes. Fetal MRI data will be used to perform 2D measurements of cortical development (sulcal depth and grading) and 3D reconstruction of fetal brain following a methodology developed in our group

to automatically measure total brain and grey and white matter volume, cortical folding parameters and cortical thickness. The MRS data obtained will be processed by linear fitting in the frequency domain (LC Model) based on metabolite basis-sets available and each metabolite will be quantified based on a reference water spectrum, as well as using metabolite ratios.

Speech discrimination task

We will implement a procedure that has been already defined (69) in order to investigate speech discrimination in fetuses with FGR at 36 weeks of gestation at the Hospital Clinic. A loudspeaker will be positioned at approximately 10cm from the mother's abdomen, typically on top of a thick pillow placed on the mother's lap. Loudspeaker will play the stimuli for the fetus at a standard intensity of 95dB (enough to pass the uterine wall). Intensity will be measured prior to testing. While sounds are played to fetus, mothers listened to a children story through noise-cancelling headphones connected to another device. Prior the stimuli, mother will do a 15-20-minute resting in order to help to minimize fetuses' movements. Different stimuli will be presented to the fetus and we will monitor continuously fetuses' heart rate with a fetal cardiotocograph, a standard tool to measure fetal well-being. We will analyze changes in fetuses' heart rate in response to sound switch.

Fetal echocardiography assessment

Fetal cardiovascular remodeling will be assessed at 32 weeks of gestation by measuring the following parameters:

- Heart area/thorax: cardiac area will be delineated in end-diastole from a 4-chamber view and divided by thoracic area in order to calculate cardio-thoracic ratio.
- Right ventricular sphericity: will be calculated by dividing the end-diastolic base to apex ventricular length by the transverse ventricular diameter measured in 2D in an apical or basal 4-chamber view (70).
- Septum thickness: will be measured from an apical or basal 4-chamber view at end-diastole.
- Pericardial effusion: we will evaluate the presence or not of pericardial effusion. It is considered physiological if <2mm and does not exceed the atrioventricular level.
- TAPSE: will be calculated using M-more real time from an apical or basal 4-chamber view, measuring the maximum displacement of the valvular ring between end-systole and end-diastole (71).
- Aortic diameter and velocity: measurement of the size of the Aortic artery at the level of the valve ring in systole. The assessment of the Aortic flow will be done by the applications of color Doppler and measurement of the peak systolic velocity (normal <120 cm/s).
- Pulmonary diameter and velocity: measurement of the size of the Pulmonary artery at the level of the valve ring in systole. The assessment of the Pulmonary flow will be done by the applications of color Doppler and measurement of the peak systolic velocity (normal <120 cm/s).
- Myocardial performance index (MPI): will be measured in a cross sectional view of the fetal thorax, in an apical projection and at the lever of the 4-chamber view of the heart (72). Briefly, the Doppler volume sample will be placed to include both the lateral wall of the ascending aorta and the mitral valve where the click corresponding to the opening and closing of the two valves can be clearly visualized. The isovolumetric contraction time (ICT), ejection time (ET) and isovolumetric relaxation time (IRT) will be calculated

using the beginning of the mitral and aortic valves clicks as landmarks and the MPI will be calculated as follow: (ICT + IRT)/ET.

- Aortic isthmus PI: will be measured either in a sagittal view of the fetal thorax with clear visualization of the aortic arch, placing the gate a few millimeters beyond the origin of the left subclavian artery; or in a cross sectional view of the fetal thorax, at the level of the three vessel and traquea view, placing the gate just at the converge of the aortic isthmus and the arterial duct (73–75).

Computerized NST

The non-stress test is an assessment tool used to evaluate fetal health through the use of electric fetal monitors that continuously record the fetal heart rate. Computerized NST provide more objective and accurate fetal information since does not depend on subjective visual inspection. This test will be done at inclusion, 28 and 36 weeks of gestation in those patients with Doppler alteration. Variables being assessed will include:

- Signal loss
- Basal heart rate
- Accelerations >10 bpm
- Accelerations >15 bpm
- Total accelerations
- Decelerations >20 missed beats
- Short term variation
- Sinusoidal pattern
- Fetal movements

Placenta pathology

After delivery, placentas will be collected and formalin fixed. After 24-48h, basic histological assessment will be carried out at the pathology service at the Hospital Sant Joan de Deu and Hospital Clínic. Tissue sections will be formalin-fixed and paraffin-embedded, followed by hematoxilina-eosin staining. Experienced placental pathologist blinded to randomization group will evaluate all placentas to ascertain the presence of placental lesions. Variables beeing assessed will include:

- Placental weight
- Maternal vascular malfusion
- Fetal vascular malfusion
- Chronic villusitis of undetermined etiology
- Chronic villusisis of probable infectious origin
- Peri-villous fibrin deposits
- Diffuse chronic intervillositis

Maternal and fetal cord blood sample analysis

At the recruitment and delivery, maternal and fetal cord blood will be collected and stored to evaluate adherence and response to DHA and Lactoferrin supplementation, by measuring biomarkers in maternal and fetal cord blood, including Lactoferrin and DHA levels. Angiogenic factors will be determined in maternal blood at the recruitment and delivery in order to relate the expected improvement to the pathophysiology of FGR. Also, at recruitment and delivery,

cytokines (TNFa, IL-6, IL-8, IL-4, IL-10) will be measured in maternal blood to evaluate the anti-inflammatory effect of the maternal supplementation.

Maternal and fetal cord blood analysis will be performed in a random subgroup of 100 women and 100 neonates.

10 mL of maternal blood will be drawn from an indwelling cannula in the brachial vein and kept in EDTA treated tubes. 10 mL of cord blood will be obtained from the umbilical vein after cord clamping at delivery and kept in EDTA treated tubes. In both cases, plasma, erythrocytes and buffy coat will be separated by centrifugation at 2500 rpm for 10 minutes at 4º C. Erythrocytes will be used to analyze the red blood cell membrane fatty acid composition. Plasma will be stored immediately at -80ºC for further analysis when patient recruitment is completed.

Red blood cell membrane fatty acid composition will be determined by chromatography. Lactoferrin levels, angiogenic factors and cytokines will be analyze using specific kits.

Postnatal follow-up

Cardiovascular assessment and IM thickness

- Systolic and diastolic blood pressure will be obtained at 6 and 24 months of corrected age by a trained physician from the brachial artery using a validated ambulatory automated Omron 5 Series device, while the infant is resting. Blood pressure percentiles will be calculated according to published reference values (76). Heart frequency will be also registered.
- At delivery and also at 6 and 24 months of corrected age, weight, height and head circumference will be measured at planned follow-up visits.

Neurodevelopmental assessment

- Bayley-III Scale of Infant and Toddler Development (77) will be performed at 6 and 24 months of corrected postnatal age at the Hospital Clínic. This scale is an individually administered instrument that assesses infant development across five domains, including cognitive, language and motor competencies. Parent reported questionnaires are incorporated into the Bayley-III scale to assess social-emotional and adaptive behaviors. Psychologists performing the evaluation will be blinded to randomization group.
- Speech discrimination task at 4-month of corrected postnatal age. The procedure will be based on a pilot study ran by the same research team lead by Nuria Sebastian-Galles at Universidad Pompeu Fabra. Infants will be presented with sequences of sounds that are consistent with their native language and sequences of sounds that are not consistent with native language. Such sounds will be short sentences or words, and will be paired with neutral visual stimuli in order to keep the infant entertained. The experiment will last maximum 10 minutes. To evaluate infants' response to speech we will use three distinct measures:
    - Electroencephalography (ECG)
    - Pupillometry using an eye-tracker (non-invasive sensor that detect the pupil size)
    - Heart rate measured with Diffuse Correlations Spectroscopy (DCS). DCS sensors will be incorporated in the ECG cap.
- Attention switching task at 4-month of corrected postnatal age. The aim of this task is to measure infants' ability to flexible switch their attention from one object to another. The procedure will be developed based on previous studies (78). Infants will be seated

in front of a computer screen equipped with an eye-tracker. On the screen there will be two boxes, then an attention getter will appear in between the two boxes to grasp infants' attention. Next, an animated toy accompanied with sounds will appear in one of the boxes.

- Vocabulary assessment at 18, 24 and 36-month of corrected postnatal age. We will assess vocabulary knowledge of infants. We will use and adaptation of the MacArthur-Bates Questionnaire (79) that has been extensively used in the BabyLab (UPF) to assess vocabulary knowledge at different ages. The questionnaire will be filled by caregivers to gather important information about the children's early language abilities such us vocabulary comprehension and production as well as gestures and grammar.
- Logical reasoning task at 19-month of corrected postnatal age. The aim of this task is investigating reasoning in infants and the basic logical representations available at 19 months. The procedure will be a logic reasoning task used in previous studies (80). Test will take from 4 to 10 minutes.
- Early Childhood Behavior Questionnaire for temperament at 24-month of corrected postnatal age. We will assess temperament using the Early Childhood Behavior Questionnaire. Caregivers will be asked to rate the frequency of some children's behavior in different situations during the previous 1 or 2 weeks, in a 7-point scale. The regular version of the questionnaire assesses 18 dimensions of temperament (activity level/energy, attentional focusing, attentional shifting, cuddliness, discomfort, fear, frustration, high-intensity pleasure, impulsivity, inhibitory control, low-intensity pleasure, motor activation, perceptual sensitivity, positive anticipation, sadness, shyness, sociability, soothability).

The Bayley scale will be performed by the postnatal follow-up platform of BCNatal's Fetal and Perinatal Medicine research group. All the other neurodevelopment assessment, as well as the speech discrimination task at 36 weeks of gestation, are optional and will be carried out in the Speech Acquisition and Perception Lab of the Universitat Pompeu Fabra Barcelona. Participants will be contacted by telephone in order to explain the expanded neurodevelopment assessment. A specific consent form will be signed in case patients decide to participate.

Variables

Socio-demographic data: birth date, both parents' socioeconomic status and educational level, pregestational weight and height, ethnicity, smoking status, parity, relevant medical history, last menstrual period.

Nutritional and lifestyle interviews: adherence to the Mediterranean diet, perceived stress scale, Pittsburgh sleep quality index, WHO-5 Well-being Index.

Maternal blood sampling: DHA and Lactoferrin levels, angiogenic factors and cytokines.

Prenatal follow up: estimated fetal weight and Doppler ultrasound measurements, neurological profile assessment (neurosonography and MRI), cardiovascular profile assessment and computerized NST assessment.

Perinatal data: mode of delivery, induction of labor, emergency cesarean section, gender, weight and height, Apgar score at 1 and 5 minutes, arterial and venous cord birth pH, pregnancy complications, exposure to corticoids, perinatal death, maternal and fetal cord blood DHA and Lactoferrin levels, maternal levels of angiogenic factors and cytokines and placental pathology.

Postnatal follow up: cardiovascular and neurodevelopmental profile assessment, anthropometrical assessment and breastfeeding assessment (type of neonatal feeding, duration of maternal breastfeeding, start of complementary diet, maternal supplements).

DATA COLLECTION AND STATISTICAL ANALYSIS

Main outcome variables

1. Bayley III scale at 24 months of age

Secondary outcomes

1. Cortical development
2. Fetal brain volume assessed by fetal MRI
3. Corpus Callosum area assessed by Neurosonography
4. Neonatal weight
5. Perinatal morbidity and mortality
6. Postnatal neurodevelopmental assessment
7. Occurrence of adverse effects

The primary analysis will be based on the intention-to-treat population. A secondary analysis will be performed according to effective treatment. Single and multiple models will be tested. Logistic regression for qualitative and linear regression for quantitative outcome (neurodevelopment test variables) will be done. We will consider a significant improvement in neurodevelopment secondary to the intervention if an improvement in at least 5 units in one of the variables being assessed in the Bayley III test is observed in comparison to the placebo group. Statistical analysis will be performed by the research team and supported by statistical services from Fundació Sant Joan de Déu. Differences in these principal variables between experimental groups (treated vs placebo groups) would be assessed by t de Student test for comparison of means between two independent samples.

Potential confounding variables that might influence the outcome of our study have been evaluated carefully. The variables identified included: corticoids administration during pregnancy for lung maturation, gestational age at delivery, mode of delivery (spontaneous vaginal delivery, instrumented vaginal delivery or cesarean section), type of delivery (labor induction or spontaneous onset), fetal distress at delivery, duration of breastfeeding, gender, socioeconomics and marital status, abuse of toxic substances and additional intake of DHA or Lactoferrin from diet or from commercial medicines. Despite randomization is expected to counteract for these potential confounders, a preliminary statistical analysis will be performed to ensure both study groups have similar baseline characteristics. At the middle of the study period, a preliminary analysis of the data will be performed. The analysis will evaluate performance of Bayley III at the 6 months of age and structural changes at the neurosonography and MRI between groups. In order to keep the study masked and not stop the randomization process, an independent researcher will pseudonymize the already included patients. Researchers could identify from which experimental group the data comes, without knowing from which individual they come.

POTENTIAL DIFFICULTIES AND LIMITATIONS

Discontinuing or modifying interventions and adherence: Since the intervention is usually well tolerated and no major side effects have been described, we do not expect to stop or modify the intervention secondary to side-effects or harms. During the intervention period, clinicians will encourage and ask directly about compliance. Finally, biomarkers for treatment compliance will be assessed by a dosage quantification of both Lactoferrin and DHA in maternal and cord blood samples.

Duration of intervention: Some included patients will deliver before finishing 4 weeks of treatment due to worsening of Doppler status. In this case, these patients will be excluded from the final analysis. In addition, in order to evaluate the effect of different durations of intervention, this variable will be assessed in a secondary analysis of the data.

Prematurity: The potential effect of prematurity in neurodevelopment can produce a bias in the results obtained. In order to explore this effect, we will apply two strategies. First, as previously mentioned, we will adjust all the statistical analysis by gestational age at delivery. In a second step, we will perform a secondary analysis of the obtained results stratifying by the moment of delivery (= 32 weeks).

Breastfeeding: As breastfeeding is one of the postnatal interventions showing demonstrated benefits in this population, we will promote breastfeeding in patients included in this study. We will collect data about breastfeeding rate and duration and all statistical analysis on neurodevelopmental tests will be adjusted by this information. In addition, a secondary analysis of the data will be performed stratifying by the duration and type (exclusive or mixt) of breastfeeding.

Loss to follow-up: Specific actions will be planned in order to minimize loss to follow-up, which is one of the potential limitations of this study. During the prenatal period, such cases are expected to be few, since diagnosis of FGR implies a close clinical monitoring by fetal ultrasound weekly. However, cases lost could increase after delivery. Therefore, the sample size has been calculated estimating a loss to follow-up rate up to 30% at 6 months of follow-up. Aiming to reduce these cases, reminders will be sent by phone and email to patients

ETHICAL ASPECTS

The project has been approved by the Institutional Review Board for clinical research (HCB/2018/1131 and PIC-29-19). The patients will be thoroughly informed prior to enrolment, and written informed consent will be obtained. This project fully complies with the relevant national, European and international ethics-related rules and professional codes of conduct, including the declaration of Helsinki in its latest version and the GCP guidelines on good clinical practice in therapeutic trials (Directive 2001/20/EC).

APPENDIX 1: study participation informed consent

Study title: FetalBrainCare

Principal investigator:

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Hospital Sant Joan de Déu (93 253 21 00)

Center: BCNatal (Hospital Clínic de Barcelona - Hospital Sant Joan de Déu)

Promotor: IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer)

INTRODUCTION

We are writing to you to invite you to participate in the clinical trial (FetalBrainCare). This study is carried out jointly with other centers in Spain (Hospital Clínic de Barcelona, Hospital Sant Joan de Déu, Hospital Sant Pau, Hospital Can Ruti, Hospital del Mar y Hospital Dexeus-Quirón). It has been approved by the Clinical Research Ethics Committee of each center in accordance with current legislation (Law 14/2007 on biomedical research). It is our intention that you receive correct and sufficient information so that you can decide whether or not to participate in this study. For this reason, read this information sheet carefully and we will clarify any doubts that may arise. In addition, you can consult with the people you deem appropriate.

VOLUNTARY PARTICIPATION

You should know that your participation in this study is voluntary and that you can decide not to participate or change your decision and withdraw your consent at any time without altering your relationship with your doctor or causing any harm to your treatment.

GENERAL DESCRIPTION OF THE STUDY

We offer you the possibility of participating in this study because we have detected in the ultrasound of the third trimester of pregnancy that your baby has an intrauterine growth restriction (IUGR). IURG is a complication that appears during pregnancy and consists of a decrease in the growth of the baby, generally secondary to a malfunction of the placenta. The malfunction of the placenta means that the placenta does not get enough blood, which means that the baby receives less nutrients affecting both its growth and the correct development of the organs, with a more important effect on the brain and the heart.

WHY ARE WE CONDUCTING THIS STUDY?

Nowadays, there is no treatment that has been shown to cure the alteration of the placenta, as well as to protect the brain and the heart from the lack of nutrients. Increasing the maternal intake of certain nutrients with protective effects on the development of the brain and the heart could constitute a fundamental pillar for the prevention of complications in these babies. In this way, with this study we want to demonstrate that with a maternal supplementation with docohexaenoic acid (DHA) and lactoferrin with fetuses diagnosed with IUGR, we are able to improve brain and cardiac development.

HOW DOES THE STUDY WORK?

We need to include a total of 304 pregnant women with the finding of an IUGR in the fetus to evaluate whether the supplementation of maternal nutrition with DHA and lactoferrin protects neurological and cardiac development in these babies. If you agree to participate, it will be determined randomly if you fall into the group of patients which will receive the supplementation (1000mf of lactoferrin + 1000mf od DHA daily orally) or if you fall into the placebo group (similar presentation to that used in the treatment group, but without containing DHA or lactoferrin). You are just as likely to receive the supplementation as placebo. Neither you nor the medical personnel treating you will know if you are in the supplementation group or the placebo group. This is the only way to answer the question of whether this treatment is really helpful in patients like you.

WHAT DO I HAVE TO DO IF I DECIDE TO PARTICIPATE IN THE STUDY?

You will take the supplementation/placebo every day from the start of the study (24-32.6 weeks) until the 37th week of pregnancy. At the beginning of the study, a maternal blood sample will be collected in addition to the usual clinical follow-up in a random subgroup of 100 women. This subgroup of patients will be randomly selected. Subsequently, you will follow the usual follow-up proposed by your medical center (medical appointments, ultrasounds, blood and urine tests

and blood pressure measurement). However, during the follow-up of the pregnancy, additional examinations will be carried out aimed at evaluating in a very specific way the neurological and cardiovascular development of the baby. For this propose, a neurosonography (ultrasound evaluation aimed at evaluating neurological development) and an echocardiography (ultrasound evaluation aimed at evaluating cardiac development) will be scheduled at Hospital Clínic or at Hospital Sant Joan de Déu at the time of inclusion in the study and during pregnancy monitoring. Additionally, an MRI will be performed to be able to asses neurological development very precisely at BCNatal around 34 weeks of gestation. In addition, both at the start of the study and at 36 weeks of gestation, you will be given questionnaires you should fill out in order to assess your lifestyle and stress level. You will be able to give birth in your center of origin. In the same subgroup of 100 patients, a sample of umbilical cord blood and maternal blood will be collected at the time of delivery. After delivery, medical data about you and your baby will be collected. In addition, the baby's neurodevelopment will be closely monitored, both at the center of origin and at Hospital Clinic or Hospital Sant Joan de Déu. At 6 and 24 months of life, a neurodevelopmental assessment will be carried out by applying a functional test called the Bayley III test. The same day that this test is performed, the baby's blood pressure will be determined. During the pregnancy and postnatal follow-up, you will be contacted by telephone by the Speech Acquisitions and Perception Group of the Universitat Pompeu Fabra in order to expand the neurodevelopmental assessment of the baby. A specific consent form will be signed in case you decide to expand this study.

Apart from completing the study visits and activities, you must also notify any adverse event that happens to you or changes in the medication. Except in an emergency, you cannot modify the medication you are taking or take other medications or "medicinal plants" without first consulting with the study doctor.

BENEFITS AND RISKS DERIVED FROM YOUR PARTICIPATION IN THE STUDY

The main Benefit expected from this study is to objectify an improvement, especially in the brain development in babies who have suffered IUGR during fetal life. However, it is possible that any benefit will be obtained for your health or for your child for you participation in this study, either due to lack of efficacy of the supplements under study or due to taking placebo. If during the course of this study definitive data are obtained on the usefulness or otherwise of the treatment, you will be informed so that you can make the decision you deem appropriate about it.

Both DHA and lactoferrin are considered dietary supplements according to the Spanish Agency for Medicines and Medical Devices (AEMPS). Both substances at the proposed doses have been shown to be safe without presenting adverse or undesirable effects. There is prior information in humans on the safety of DHA during pregnancy and lactation. In the case of lactoferrin, information on safety in pregnancy and lactation comes from animal studies. Even the administration of these substances at higher doses that those proposed in this study have been

used for other problems without evidence of significant undesirable effects, although some patients and at very high doses have presented gastrointestinal discomfort (stomach or intestinal discomfort, diarrhea and constipation) and some episodes of skin rash. In this case, you can contact you doctor to assess these discomforts and whether or not to continue the treatment.

In addition, no risk is expected from the tests performed during the study. Both obstetric ultrasound and Tesla MRI have proven to be safe and not harmful, which is why today they are routine tests in obstetric monitoring when indicated.

INSURANCE

If you suffer any damage from participating in the study, all means will be made available to remedy it. In addition, the promoter has contracted a Civil Liability Insurance according to current legislation that covers any eventuality, providing compensation in case of impairment of your heath or injuries that may occur in relation to your participation in the study, provided that these are not consequence of the disease itself.

WHAT HAPPENS WITH THE INFORMATON WE COLLECT?

The Hospital Clínic, with Tax Identification Code 0802070C, as responsible for the processing of you data, informs you that the treatment, communication and transfer of personal data of all participants will comply with the EU Regulation 2016/679 of the European Parliament and the Council of April 27th 2016 regarding the protection of natural persons with reference to the processing of personal data and the free circulation of data and to the Organic Law 3/2018 of December 5th on Protection of Personal Data and Guarantee of digital rights.

Data collected for this study will be identified only by a code, so any information identifying the participants will be included. Only the study investigators and his collaborators with specific permission will be able to relate your data collected in the study with your medical history.

Your identity will not be available to any other person except for a medical emergency or legal requirement. The health authorities, the Research Ethics Committee and personnel authorized by the study promoter will have access to your personal information, when necessary to verify data and study procedures, but always maintaining confidentiality in accordance with current legislation.

Only coded data Will be transferred to third parties and other countries, which in no case will contain information that can directly identify the participant (such as name and surname, initials, address, social security number, etc.). In case this transfer occurs, it would be for the same purpose as described in the study and guaranteeing confidentiality.

If a transfer of encrypted data is made outside the EU, either to entities related to the hospital center where you participate, to service providers or researchers who collaborate with your doctor, your data will be protected by safeguards such as contracts or other mechanisms established by the data protection authorities.

In addition to the rights that the previous legislations already contemplated (access, modification, opposition and data cancellation, deletion in the new Regulation) you can now also limit the processing of data that are incorrect, request a copy or request that data to be transferred to a third party (portability). To exercise these rights, or if you want to know more about confidentiality. You should contact the main researcher of the study or the Data Protection Delegate or the Hospital Clínic de Barcelona through protecciodades@clinic.cat. You have also the right to contact the Data Protection Agency if you are not satisfied.

Data already collected cannot be delete even if you leave the study to ensure the validity of the research and to comply with legal duties and drug authorization requirements. But no new data will be collected if you decide to stop participating.

The researcher and the sponsor are obliged to keep the data collected for the study for at least 5 years after its completion. Subsequently, the personal information will only be kept by the center for the care or your health and by the sponsor for other scientific research purposes if the patient has given its consent to do so, and if this is permitted by law and applicable ethical requirements.

ECONOMIC COMPENSATION

You will not be charged for your participation in the study and for the study drugs. You will not receive any financial compensation for your participation in this study.

WHAT WILL BE DONE WITH THE BLOOD SAMPLES?

At the time of inclusion in the study, a maternal blood sample will be drawn in order to determine baseline levels of DHA and lactoferrin as well as angiogenic factors and certain cytokines. At the time of delivery, another sample of maternal blood will be taken to evaluate the levels of DHA and lactoferrin achieved at the end of the study, the angiogenic factors and the level of certain cytokines. Blood will also be collected from the umbilical cord to be able to evaluate the levels of DHA and lactoferrin in the baby's blood, without having to directly prick the baby. These samples are biological samples for which all assumptions will be met in accordance with the Biomedical Research Lay 14/2007 and RD 1716/2011 of November 18th, which establishes the basic requirements for authorization and operation of the biobanks for biomedical research purposes and the treatment of biological samples of human origin. By signing this document, you agree the use of the samples collected for the purposes of this study.

The simples will be kept stored in the Biobank-IDIBAPS of Maternal-Fetal Medicine at Hospital Clínic until they are used for the purposes of this study. Once completed, the remaining samples will be Destroyer, unless you sign a specific consent form in order to be stored and used in future research (this will be provided separately).

A code will be used to identify your samples and no data that could reveal your identity will be used. Only the study doctor and his collaborators will be able to associate the samples with you.

The data derived from the use of these samples will be treated in the same way as the rest of the data obtained during this study.

The transfer of biological samples for this study is free and voluntary. This means that you will not have rights to possible commercial benefits of the findings that could be derived from de results of biomedical research.

If relevant information is obtained that could affect you or your family members health, you will be notified. If it is necessary to contact you, the data that appears in your medical history will be used. However, your right to not receive that information will be respected. In that case, you have to check the specific box found on the consent form.

OTHER RELEVANT INFORMATION

Any new information regarding the treatment used in the study that may affect your willingness to participate or that is discovered during your participation, will be communicated to you by your doctor as soon as possible.

If you decide to withdraw your consent to participate in this study, no new data will be added to the database and you can demand the destruction of all identifiable samples previously retained to avoid further analysis.

You should also know that you can be excluded from the study if the promoter and/or the study investigators consider it appropriate, either for safety reasons, for any adverse event that occurs and is considered to be related to your participation in the study or because they consider that you are not complying with established procedures. In either case, you will receive an adequate explanation of the reason for your withdrawal from the study.

By signing the attached consent form, you agree to comply with the study procedures that have been set forth to you.

Participant consent form sheet

Study tittle: FetalBrainCare

Version 6 (November 2022)

I, *(name and surname of the participant)* _____

- I have read the informational sheet given to me about the study.

- I have been able to ask questions about the study.

- I have received enough information about the study.

- I have spoken with: (name of researcher) _____

- I understand that my participation is voluntary.

- I understand that I can withdraw from the study:

    - Whenever I want.

    - Without having to explain anything.

    - Without this affecting my medical care.

- In accordance with the EU Regulation 2016/679 of the European Parliament and the Council of April 27th 2016 regarding the protection of natural persons with reference to the processing of personal data and the free circulation of data and to the Organic Law 3/2018 of December 5th on Protection of Personal Data and Guarantee of digital rights, I declare to have been informed of the existence of storage of processing of personal data, of the purpose of the collection of that data and of the recipients of the information.

Given this information that the Data Controller has given to me, and having understood it, I offer my consent to the treatment of:

☐ My personal data to carry out the research project.

☐ My personal data to carry out research projects related to the present or in the same research area.

I freely give my consent to participate in the study.

Signature of participant	Signature of researcher
Date: ____/____/____	Date: ____/____/____

I would like to be informed about any information derived from the research that may be relevant to my health:

☐ YES ☐ NO

I would like to be contacted by Pompeu Fabra University to complete the postnatal neurological follow-up:

☐ YES ☐ NO

Signature of participant	Signature of researcher
Date: ____/____/____	Date: ____/____/____

BIBLIOGRAPHY

1. Arcangeli T, Thilaganathan B, Hooper R, Khan KS, Bhide A. Neurodevelopmental delay in small babies at term: A systematic review [Internet]. Vol. 40, Ultrasound in Obstetrics and Gynecology. Ultrasound Obstet Gynecol; 2012 [cited 2020 Jul 6]. p. 267–75. Available from: https://pubmed.ncbi.nlm.nih.gov/22302630/

2. MacKay DF, Smith GCS, Dobbie R, Cooper SA, Pell JP. Obstetric factors and different causes of special educational need: Retrospective cohort study of 407 503 schoolchildren. BJOG An Int J Obstet Gynaecol [Internet]. 2013 [cited 2020 Jul 6];120(3):297–308. Available from: https://pubmed.ncbi.nlm.nih.gov/23189965/

3. Moore GS, Kneitel AW, Walker CK, Gilbert WM, Xing G. Autism risk in small- and large-for-gestational-age infants. In: American Journal of Obstetrics and Gynecology [Internet]. Mosby Inc.; 2012 [cited 2020 Jul 6]. p. 314.e1-314.e9. Available from: https://pubmed.ncbi.nlm.nih.gov/22464070/

4. Heinonen K, Räikkönen K, Pesonen AK, Andersson S, Kajantie E, Eriksson JG, et al. Behavioural symptoms of attention deficit/hyperactivity disorder in preterm and term children born small and appropriate for gestational age: A longitudinal study. BMC Pediatr [Internet]. 2010 Dec 15 [cited 2020 Jul 6];10. Available from: https://pubmed.ncbi.nlm.nih.gov/21159164/

5. Egaña-Ugrinovic G, Sanz-Cortes M, Figueras F, Bargalló N, Gratacós E. Differences in cortical development assessed by fetal MRI in late-onset intrauterine growth restriction. Am J Obstet Gynecol [Internet]. 2013 [cited 2020 Jul 6];209(2):126.e1-126.e8. Available from: https://pubmed.ncbi.nlm.nih.gov/23583839/

6. Padilla N, Falcón C, Sanz-Cortés M, Figueras F, Bargallo N, Crispi F, et al. Differential effects of intrauterine growth restriction on brain structure and development in preterm infants: A magnetic resonance imaging study. Brain Res [Internet]. 2011 Mar 25 [cited 2020 Jul 6];1382:98–108. Available from: https://pubmed.ncbi.nlm.nih.gov/21255560/

7. Martinussen M, Fischl B, Larsson HB, Skranes J, Kulseng S, Vangberg TR, et al. Cerebral cortex thickness in 15-year-old adolescents with low birth weight measured by an automated MRI-based method. Brain [Internet]. 2005 [cited 2020 Jul 6];128:2588–96. Available from: https://academic.oup.com/brain/article-abstract/128/11/2588/339568

8. Rao MR, Hediger ML, Levine RJ, Naficy AB. Effect of breastfeeding on cognitive development of infants born small for gestational age. Acta Paediatr Int J Paediatr [Internet]. 2002 Jan 1 [cited 2020 Jul 6];91(3):267–74. Available from: https://pubmed.ncbi.nlm.nih.gov/12022297/

9. Illa M, Brito V, Pla L, Eixarch E, Arbat-Plana A, Batallé D, et al. Early Environmental Enrichment Enhances Abnormal Brain Connectivity in a Rabbit Model of Intrauterine Growth Restriction. Fetal Diagn Ther [Internet]. 2018 Oct 1 [cited 2020 Jul 6];44(3):184–93. Available from: https://pubmed.ncbi.nlm.nih.gov/29020672/

10. Andersen SL. Trajectories of brain development: Point of vulnerability or window of opportunity? In: Neuroscience and Biobehavioral Reviews [Internet]. Elsevier Ltd; 2003 [cited 2020 Jul 6]. p. 3–18. Available from: https://pubmed.ncbi.nlm.nih.gov/12732219/

11. Innis SM. Essential fatty acid transfer and fetal development. Placenta [Internet]. 2005 Apr [cited 2020 Jul 6];26(SUPPL.). Available from: https://pubmed.ncbi.nlm.nih.gov/15837071/

12. Innis SM. Fatty acids and early human development. Early Hum Dev [Internet]. 2007 Dec [cited 2020 Jul 6];83(12):761–6. Available from: https://pubmed.ncbi.nlm.nih.gov/17920214/

13. Arteaga O, Revuelta M, Urigüen L, Martínez-Millán L, Hilario E, Álvarez A. Docosahexaenoic Acid Reduces Cerebral Damage and Ameliorates Long-Term Cognitive Impairments Caused by Neonatal Hypoxia–Ischemia in Rats. Mol Neurobiol [Internet]. 2017 Nov 1 [cited 2020 Jul 6];54(9):7137–55. Available from: https://pubmed.ncbi.nlm.nih.gov/27796751/

14. Wang B. Molecular Determinants of Milk Lactoferrin as a Bioactive Compound in Early Neurodevelopment and Cognition. J Pediatr [Internet]. 2016 Jun 1 [cited 2020 Jul 6];173:S29–36. Available from: https://pubmed.ncbi.nlm.nih.gov/27234408/

15. Somm E, Larvaron P, Van De Looij Y, Toulotte A, Chatagner A, Faure M, et al. Protective effects of maternal nutritional supplementation with lactoferrin on growth and brain metabolism. Pediatr Res [Internet]. 2014 [cited 2020 Jul 6];75(1):51–61. Available from: https://pubmed.ncbi.nlm.nih.gov/24213624/

16. van de Looij Y, Ginet V, Chatagner A, Toulotte A, Somm E, Hüppi PS, et al. Lactoferrin during lactation protects the immature hypoxic-ischemic rat brain. Ann Clin Transl Neurol [Internet]. 2014 Dec 1 [cited 2020 Jul 6];1(12):955–67. Available from: https://pubmed.ncbi.nlm.nih.gov/25574471/

17. McNamara RK, Asch RH, Lindquist DM, Krikorian R. Role of polyunsaturated fatty acids in human brain structure and function across the lifespan: An update on neuroimaging findings [Internet]. Vol. 136, Prostaglandins Leukotrienes and Essential Fatty Acids. Churchill Livingstone; 2018 [cited 2020 Jul 6]. p. 23–34. Available from: https://pubmed.ncbi.nlm.nih.gov/28529008/

18. Joshi K, Lad S, Kale M, Patwardhan B, Mahadik SP, Patni B, et al. Supplementation with flax oil and vitamin C improves the outcome of Attention Deficit Hyperactivity Disorder (ADHD). Prostaglandins Leukot Essent Fat Acids [Internet]. 2006 Jan [cited 2020 Jul 6];74(1):17–21. Available from: https://pubmed.ncbi.nlm.nih.gov/16314082/

19. Strømmen K, Blakstad EW, Moltu SJ, Almaas AN, Westerberg AC, Amlien IK, et al. Enhanced nutrient supply to very low birth weight infants is associated with improved white matter maturation and head growth. Neonatology [Internet]. 2015 Dec 17 [cited 2020 Jul 6];107(1):68–75. Available from: https://pubmed.ncbi.nlm.nih.gov/25401387/

20. Waworuntu R V., Hanania T, Boikess SR, Rex CS, Berg BM. Early life diet containing prebiotics and bioactive whey protein fractions increased dendritic spine density of rat hippocampal neurons. Int J Dev Neurosci [Internet]. 2016 Dec 1 [cited 2020 Jul 6];55:28–33. Available from: https://pubmed.ncbi.nlm.nih.gov/27603970/

21. Chang PKY, Khatchadourian A, McKinney AA, Maysinger D. Docosahexaenoic acid (DHA): A modulator of microglia activity and dendritic spine morphology. J Neuroinflammation [Internet]. 2015 Feb 22 [cited 2020 Jul 6];12(1). Available from: https://pubmed.ncbi.nlm.nih.gov/25889069/

22. Meldrum S, Simmer K. Docosahexaenoic acid and neurodevelopmental outcomes of term infants. Ann Nutr Metab [Internet]. 2016 Nov 1 [cited 2020 Jul 6];69(1):23–8. Available from: https://pubmed.ncbi.nlm.nih.gov/27842311/

23. Lauritzen L, Brambilla P, Mazzocchi A, Harsløf LBS, Ciappolino V, Agostoni C. DHA effects in brain development and function [Internet]. Vol. 8, Nutrients. MDPI AG; 2016 [cited 2020 Jul 6]. Available from: https://pubmed.ncbi.nlm.nih.gov/26742060/

24. Ginet V, van de Looij Y, Petrenko V, Toulotte A, Kiss J, Hüppi PS, et al. Lactoferrin during lactation reduces lipopolysaccharide-induced brain injury. BioFactors [Internet]. 2016 May 1 [cited 2020 Jul 6];42(3):323–36. Available from: https://pubmed.ncbi.nlm.nih.gov/27313089/

25. Chen Y, Zheng Z, Zhu X, Shi Y, Tian D, Zhao F, et al. Lactoferrin Promotes Early Neurodevelopment and Cognition in Postnatal Piglets by Upregulating the BDNF Signaling Pathway and Polysialylation. Mol Neurobiol [Internet]. 2015 Aug 25 [cited 2020 Jul 6];52(1):256–69. Available from: https://pubmed.ncbi.nlm.nih.gov/25146846/

26. Rousseau E, Michel PP, Hirsch EC. The iron-binding protein lactoferrin protects vulnerable dopamine neurons from degeneration by preserving mitochondrial calcium homeostasis. Mol Pharmacol [Internet]. 2013 Dec [cited 2020 Jul 6];84(6):888–98. Available from: https://pubmed.ncbi.nlm.nih.gov/24077968/

27. Zugno AI, Chipindo HL, Volpato AM, Budni J, Steckert A V., de Oliveira MB, et al. Omega-3 prevents behavior response and brain oxidative damage in the ketamine model of schizophrenia.

Neuroscience [Internet]. 2014 Feb 14 [cited 2020 Jul 6];259:223–31. Available from: https://pubmed.ncbi.nlm.nih.gov/24316471/

28. Hernandez-Andrade E, Jansson T, Figueroa-Diesel H, Rangel-Nava H, Acosta-Rojas R, Gratacós E. Evaluation of fetal regional cerebral blood perfusion using power Doppler ultrasound and the estimation of fractional moving blood volume. Ultrasound Obstet Gynecol [Internet]. 2007 May [cited 2020 Jul 6];29(5):556–61. Available from: https://pubmed.ncbi.nlm.nih.gov/17444567/

29. Hernandez-Andrade E, Figueroa-Diesel H, Jansson T, Rangel-Nava H, Gratacos E. Changes in regional fetal cerebral blood flow perfusion in relation to hemodynamic deterioration in severely growth-restricted fetuses. Ultrasound Obstet Gynecol [Internet]. 2008 Jul [cited 2020 Jul 6];32(1):71–6. Available from: https://pubmed.ncbi.nlm.nih.gov/18570240/

30. Eixarch E, Meler E, Iraola A, Illa M, Crispi F, Hernandez-Andrade E, et al. Neurodevelopmental outcome in 2-year-old infants who were small-for-gestational age term fetuses with cerebral blood flow redistribution. Ultrasound Obstet Gynecol [Internet]. 2008 Dec [cited 2020 Jul 6];32(7):894–9. Available from: https://pubmed.ncbi.nlm.nih.gov/19035538/

31. Figueras F, Eixarch E, Meler E, Iraola A, Figueras J, Puerto B, et al. Small-for-gestational-age fetuses with normal umbilical artery Doppler have suboptimal perinatal and neurodevelopmental outcome. Eur J Obstet Gynecol Reprod Biol [Internet]. 2008 [cited 2020 Jul 6];136(1):34–8. Available from: https://pubmed.ncbi.nlm.nih.gov/17434250/

32. Figueras F, Oros D, Cruz-Martinez R, Padilla N, Hernandez-Andrade E, Botet F, et al. Neurobehavior in term, small-for-gestational age infants with normal placental function. Pediatrics [Internet]. 2009 Nov [cited 2020 Jul 6];124(5). Available from: https://pubmed.ncbi.nlm.nih.gov/19858151/

33. Esteban FJ, Padilla N, Sanz-Cortés M, de Miras JR, Bargalló N, Villoslada P, et al. Fractal-dimension analysis detects cerebral changes in preterm infants with and without intrauterine growth restriction. Neuroimage [Internet]. 2010 Dec [cited 2020 Jul 6];53(4):1225–32. Available from: https://pubmed.ncbi.nlm.nih.gov/20633658/

34. Batalle D, Eixarch E, Figueras F, Muñoz-Moreno E, Bargallo N, Illa M, et al. Altered small-world topology of structural brain networks in infants with intrauterine growth restriction and its association with later neurodevelopmental outcome. Neuroimage [Internet]. 2012 Apr 2 [cited 2020 Jul 6];60(2):1352–66. Available from: https://pubmed.ncbi.nlm.nih.gov/22281673/

35. Batalle D, Muñoz-Moreno E, Figueras F, Bargallo N, Eixarch E, Gratacos E. Normalization of similarity-based individual brain networks from gray matter MRI and its association with neurodevelopment in infants with intrauterine growth restriction. Neuroimage [Internet]. 2013 Dec [cited 2020 Jul 6];83:901–11. Available from: https://pubmed.ncbi.nlm.nih.gov/23886985/

36. Batalle D, Muñoz-Moreno E, Tornador C, Bargallo N, Deco G, Eixarch E, et al. Altered resting-state whole-brain functional networks of neonates with intrauterine growth restriction. Cortex [Internet]. 2016 Apr 1 [cited 2020 Jul 6];77:119–31. Available from: https://pubmed.ncbi.nlm.nih.gov/26927726/

37. Eixarch E, Muñoz-Moreno E, Bargallo N, Batalle D, Gratacos E. Motor and cortico-striatal-thalamic connectivity alterations in intrauterine growth restriction Presented in part at the 22nd World Congress on Ultrasound in Obstetrics and Gynaecology, Copenhagen, Denmark, September 9-12, 2012. Am J Obstet Gynecol [Internet]. 2016 Jun 1 [cited 2020 Jul 6];214(6):725.e1-725.e9. Available from: https://pubmed.ncbi.nlm.nih.gov/26719213/

38. Simões R V., Muñoz-Moreno E, Cruz-Lemini M, Eixarch E, Bargalló N, Sanz-Cortés M, et al. Brain metabolite alterations in infants born preterm with intrauterine growth restriction: association with structural changes and neurodevelopmental outcome. Am J Obstet Gynecol [Internet]. 2017 Jan 1 [cited 2020 Jul 6];216(1):62.e1-62.e14. Available from: https://pubmed.ncbi.nlm.nih.gov/27667762/

39. Egaña-Ugrinovic G, Sanz-Cortés M, Couve-Pérez C, Figueras F, Gratacós E. Corpus callosum differences assessed by fetal MRI in late-onset intrauterine growth restriction and its association

with neurobehavior. Prenat Diagn [Internet]. 2014 [cited 2020 Jul 6];34(9):843–9. Available from: https://pubmed.ncbi.nlm.nih.gov/24711150/

40. Egaña-Ugrinovic G, Sanz-Cortes M, Figueras F, Couve-Perez C, Gratacõs E. Fetal MRI insular cortical morphometry and its association with neurobehavior in late-onset small-for-gestational-age fetuses. Ultrasound Obstet Gynecol [Internet]. 2014 [cited 2020 Jul 6];44(3):322–9. Available from: https://pubmed.ncbi.nlm.nih.gov/24616027/

41. Eganã-Ugrinovic G, Savchev S, Bazán-Arcos C, Puerto B, Gratacos E, Sanz-Cortés M. Neurosonographic assessment of the corpus callosum as imaging biomarker of abnormal neurodevelopment in late-onset fetal growth restriction. Fetal Diagn Ther [Internet]. 2015 May 28 [cited 2020 Jul 6];37(4):281–8. Available from: https://pubmed.ncbi.nlm.nih.gov/25659952/

42. Sanz-Cortes M, Egaña-Ugrinovic G, Zupan R, Figueras F, Gratacos E. Brainstem and cerebellar differences and their association with neurobehavior in term small-for-gestational-age fetuses assessed by fetal MRI. Am J Obstet Gynecol [Internet]. 2014 [cited 2020 Jul 6];210(5):452.e1-452.e8. Available from: https://pubmed.ncbi.nlm.nih.gov/24315862/

43. Sanz-Cortes M, Simoes R V., Bargallo N, Masoller N, Figueras F, Gratacos E. Proton magnetic resonance spectroscopy assessment of fetal brain metabolism in late-onset “small for gestational age” versus “intrauterine growth restriction” fetuses. Fetal Diagn Ther [Internet]. 2014 [cited 2020 Jul 6];37(2):108–16. Available from: https://pubmed.ncbi.nlm.nih.gov/25115414/

44. Eixarch E, Figueras F, Hernández-Andrade E, Crispi F, Nadal A, Torre I, et al. An experimental model of fetal growth restriction based on selective ligature of uteroplacental vessels in the pregnant rabbit. Fetal Diagn Ther [Internet]. 2009 Dec [cited 2020 Jul 6];26(4):203–11. Available from: https://pubmed.ncbi.nlm.nih.gov/19955698/

45. Eixarch E, Hernandez-Andrade E, Crispi F, Illa M, Torre I, Figueras F, et al. Impact on fetal mortality and cardiovascular Doppler of selective ligature of uteroplacental vessels compared with undernutrition in a rabbit model of intrauterine growth restriction. Placenta [Internet]. 2011 Apr [cited 2020 Jul 6];32(4):304–9. Available from: https://pubmed.ncbi.nlm.nih.gov/21334065/

46. Eixarch E, Batalle D, Illa M, Muñoz-Moreno E, Arbat-Plana A, Amat-Roldan I, et al. Neonatal neurobehavior and diffusion MRI changes in brain reorganization due to intrauterine growth restriction in a rabbit model. PLoS One [Internet]. 2012 Feb 8 [cited 2020 Jul 6];7(2). Available from: https://pubmed.ncbi.nlm.nih.gov/22347486/

47. van Vliet E, Eixarch E, Illa M, Arbat-Plana A, González-Tendero A, Hogberg HT, et al. Metabolomics Reveals Metabolic Alterations by Intrauterine Growth Restriction in the Fetal Rabbit Brain. PLoS One [Internet]. 2013 May 27 [cited 2020 Jul 6];8(5). Available from: https://pubmed.ncbi.nlm.nih.gov/23724060/

48. Illa M, Eixarch E, Batalle D, Arbat-Plana A, Muñoz-Moreno E, Figueras F, et al. Long-Term Functional Outcomes and Correlation with Regional Brain Connectivity by MRI Diffusion Tractography Metrics in a Near-Term Rabbit Model of Intrauterine Growth Restriction. PLoS One [Internet]. 2013 Oct 15 [cited 2020 Jul 6];8(10). Available from: https://pubmed.ncbi.nlm.nih.gov/24143189/

49. Pla L, Illa M, Loreiro C, Lopez MC, Vázquez-Aristizabal P, Kühne BA, et al. Structural Brain Changes during the Neonatal Period in a Rabbit Model of Intrauterine Growth Restriction. Dev Neurosci [Internet]. 2020 [cited 2021 Mar 24];42(5–6):217–29. Available from: https://www.karger.com/Article/FullText/512948

50. The Investigation and Management of the Small-for-Gestational-Age Fetus. 2013.

51. Nast I, Bolten M, Meinlschmidt G, Hellhammer DH. How to measure prenatal stress? A systematic review of psychometric instruments to assess psychosocial stress during pregnancy [Internet]. Vol. 27, Paediatric and Perinatal Epidemiology. Paediatr Perinat Epidemiol; 2013 [cited 2020 Jul 6]. p. 313–22. Available from: https://pubmed.ncbi.nlm.nih.gov/23772932/

52. Julian LJ. Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and

Hospital Anxiety and Depression Scale-Anxiety (HADS-A). Arthritis Care Res [Internet]. 2011 Nov [cited 2020 Jul 6];63(SUPPL. 11). Available from: https://pubmed.ncbi.nlm.nih.gov/22588767/

53. Mindell JA, Cook RA, Nikolovski J. Sleep patterns and sleep disturbances across pregnancy. Sleep Med [Internet]. 2015 Apr 1 [cited 2020 Jul 6];16(4):483–8. Available from: https://pubmed.ncbi.nlm.nih.gov/25666847/

54. Okun ML, Schetter CD, Glynn LM. Poor Sleep Quality is Associated with Preterm Birth. Sleep [Internet]. 2011 Nov [cited 2020 Jul 6];34(11):1493–8. Available from: https://pubmed.ncbi.nlm.nih.gov/22043120/

55. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements-A prospective study. Am J Obstet Gynecol [Internet]. 1985 Feb 1 [cited 2020 Jul 6];151(3):333–7. Available from: https://pubmed.ncbi.nlm.nih.gov/3881966/

56. Arduini D, Rizzo G. Normal values of pulsatility index from fetal vessels: A cross-sectional study on 1556 healthy fetuses. J Perinat Med [Internet]. 1990 [cited 2020 Jul 6];18(3):165–72. Available from: https://pubmed.ncbi.nlm.nih.gov/2200862/

57. Figueras F, Fernandez S, Eixarch E, Gomez O, Martinez JM, Puerto B, et al. Middle cerebral artery pulsatility index: Reliability at different sampling sites. Ultrasound Obstet Gynecol [Internet]. 2006 Nov [cited 2020 Jul 6];28(6):809–13. Available from: https://pubmed.ncbi.nlm.nih.gov/17019746/

58. Forouzan I, Tian ZY. Fetal middle cerebral artery blood flow velocities in pregnancies complicated by intrauterine growth restriction and extreme abnormality in umbilical artery Doppler velocity. Am J Perinatol [Internet]. 1996 [cited 2020 Jul 6];13(3):139–42. Available from: https://pubmed.ncbi.nlm.nih.gov/8688102/

59. Crimmins S, Desai A, Block-Abraham D, Berg C, Gembruch U, Baschat AA. A comparison of Doppler and biophysical findings between liveborn and stillborn growth-restricted fetuses. Am J Obstet Gynecol [Internet]. 2014 Dec 1 [cited 2020 Jul 6];211(6):669.e1-669.e10. Available from: https://pubmed.ncbi.nlm.nih.gov/24931475/

60. Gramellini D, Folli MC, Raboni S, Vadora E, Merialdi A. Cerebral-umbilical Doppler ratio as a predictor of adverse perinatal outcome. Obstet Gynecol [Internet]. 1992 [cited 2020 Jul 6];79(3):416–20. Available from: https://pubmed.ncbi.nlm.nih.gov/1738525/

61. J W Wladimiroff, J A vd Wijngaard, S Degani, M J Noordam, J van Eyck HMT. Cerebral and Umbilical Arterial Blood Flow Velocity Waveforms in Normal and Growth-Retarded Pregnancies. Obs Gynecol. 1987;69(5):705–9.

62. Baschat AA, Gembruch U. The cerebroplacental Doppler ratio revisited. Ultrasound Obstet Gynecol [Internet]. 2003 Feb 1 [cited 2020 Jul 6];21(2):124–7. Available from: https://pubmed-ncbi-nlm-nih-gov.sire.ub.edu/12601831/

63. Hecher K, Campbell S, Snijders R, Nicolaides K. Reference ranges for fetal venous and atrioventricular blood flow parameters. Ultrasound Obstet Gynecol [Internet]. 1994 [cited 2020 Jul 6];4(5):381–90. Available from: https://pubmed-ncbi-nlm-nih-gov.sire.ub.edu/12797146/

64. Gómez O, Figueras F, Fernández S, Bennasar M, Martínez JM, Puerto B, et al. Reference ranges for uterine artery mean pulsatility index at 11-41 weeks of gestation. Ultrasound Obstet Gynecol [Internet]. 2008 Aug [cited 2020 Jul 6];32(2):128–32. Available from: https://pubmed-ncbi-nlm-nih-gov.sire.ub.edu/18457355/

65. Paladini D, Malinger G, Monteagudo A, Pilu G, Timor-Tritsch I, Toi A. Sonographic examination of the fetal central nervous system: Guidelines for performing the “basic examination” and the “fetal neurosonogram” [Internet]. Vol. 29, Ultrasound in Obstetrics and Gynecology. Ultrasound Obstet Gynecol; 2007 [cited 2020 Jul 6]. p. 109–16. Available from: https://pubmed-ncbi-nlm-nih-gov.sire.ub.edu/17200992/

66. Malinger G, Ben-Sira L, Lev D, Ben-Aroya Z, Kidron D, Lerman-Sagie T. Fetal brain imaging: A

comparison between magnetic resonance imaging and dedicated neurosonography. Ultrasound Obstet Gynecol [Internet]. 2004 Apr [cited 2020 Jul 6];23(4):333–40. Available from: https://pubmed-ncbi-nlm-nih-gov.sire.ub.edu/15065181/

67. Pistorius LR, Stoutenbeek P, Groenendaal F, De Vries L, Manten G, Mulder E, et al. Grade and symmetry of normal fetal cortical development: A longitudinal two- and three-dimensional ultrasound study. Ultrasound Obstet Gynecol [Internet]. 2010 Dec [cited 2020 Jul 6];36(6):700–8. Available from: https://pubmed-ncbi-nlm-nih-gov.sire.ub.edu/20521241/

68. SF W. Hand and sex differences in the isthmus and genu of the human corpus callosum. A postmortem morphological study. Brain. 1989;112:799–835.

69. Weikum WM, Oberlander TF, Hensch TK, Werker JF. Prenatal exposure to antidepressants and depressed maternal mood alter trajectory of infant speech perception. Proc Natl Acad Sci U S A [Internet]. 2012 Oct 16 [cited 2020 Jul 10];109(SUPPL.2):17221–7. Available from: https://pubmed.ncbi.nlm.nih.gov/23045665/

70. Crispi F, Bijnens B, Figueras F, Bartrons J, Eixarch E, Le Noble F, et al. Fetal growth restriction results in remodeled and less efficient hearts in children. Circulation. 2010;121(22):2427–36.

71. Gardiner HM, Pasquini L, Wolfenden J, Barlow A, Li W, Kulinskaya E, et al. Myocardial tissue Doppler and long axis function in the fetal heart. Int J Cardiol [Internet]. 2006 Oct 26 [cited 2020 Jul 10];113(1):39–47. Available from: https://pubmed-ncbi-nlm-nih-gov.sire.ub.edu/16360223/

72. Hernandez-Andrade E, Benavides-Serralde JA, Cruz-Martinez R, Welsh A, Mancilla-Ramirez J. Evaluation of conventional doppler fetal cardiac function parameters: E/A ratios, outflow tracts, and myocardial performance index [Internet]. Vol. 32, Fetal Diagnosis and Therapy. Fetal Diagn Ther; 2012 [cited 2020 Jul 10]. p. 22–9. Available from: https://pubmed-ncbi-nlm-nih-gov.sire.ub.edu/22677618/

73. Del Río M, Martínez JM, Figueras F, López M, Palacio M, Gómez O, et al. Reference ranges for Doppler parameters of the fetal aortic isthmus during the second half of pregnancy. Ultrasound Obstet Gynecol [Internet]. 2006 Jul [cited 2020 Jul 10];28(1):71–6. Available from: https://pubmed-ncbi-nlm-nih-gov.sire.ub.edu/16795125/

74. Rizzo G, Capponi A, Vendola M, Pietrolucci ME, Arduini D. Use of the 3-vessel view to record Doppler velocity waveforms from the aortic isthmus in normally grown and growth-restricted fetuses: Comparison with the long aortic arch view. J Ultrasound Med [Internet]. 2008 Nov 1 [cited 2020 Jul 10];27(11):1617–22. Available from: https://pubmed-ncbi-nlm-nih-gov.sire.ub.edu/18946101/

75. Fouron JC, Gosselin J, Amiel-Tison C, Infante-Rivard C, Fouron C, Skoll A, et al. Correlation between prenatal velocity waveforms in the aortic isthmus and neurodevelopmental outcome between the ages of 2 and 4 years. Am J Obstet Gynecol [Internet]. 2001 [cited 2020 Jul 10];184(4):630–6. Available from: https://pubmed-ncbi-nlm-nih-gov.sire.ub.edu/11262464/

76. De Jesus JM. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: Summary report [Internet]. Vol. 128, Pediatrics. American Academy of Pediatrics; 2011 [cited 2020 Jul 10]. Available from: https://pubmed-ncbi-nlm-nih-gov.sire.ub.edu/22084329/

77. Macha T, Petermann F. Bayley Scales of Infant and Toddler Development, Third Edition – Deutsche Fassung. http://dx.doi.org/101024/1661-4747/a000232 [Internet]. 2015 May 11 [cited 2020 Jul 10]; Available from: https://econtent.hogrefe.com/doi/abs/10.1024/1661-4747/a000232

78. Conejero Á, Rueda MR. Infant temperament and family socio-economic status in relation to the emergence of attention regulation. Sci Rep [Internet]. 2018 Dec 1 [cited 2020 Jul 12];8(1). Available from: https://pubmed.ncbi.nlm.nih.gov/30046121/

79. Mariscal S, López-Ornat S, Gallego C, Gallo P, Karousou A, Martínez M, et al. La evaluación del desarrollo comunicativo y lingüístico mediante la versión española de los inventarios MacArthur-Bates [Internet]. Vol. 19, Psicothema. 2007 [cited 2020 Jul 12]. Available from:

www.psicothema.com

80. Cesana-Arlotti N, Martín A, Téglás E, Vorobyova L, Cetnarski R, Bonatti LL. Precursors of logical reasoning in preverbal human infants. Science (80-) [Internet]. 2018 Mar 16 [cited 2020 Jul 12];359(6381):1263–6. Available from: https://pubmed.ncbi.nlm.nih.gov/29590076/