Brave Project

Effects of maternal supplementation with probiotics and omega-3 fatty acids on fetal brain growth and infant development: A double-blind, randomized trial

Statistical Analysis Plan

v1.0
2025-08-18

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CONTENTS

List of Abbreviations

AE	Adverse Event
AOIs	Area of interests
Bayley-III	Bayley Scales of Infant and Toddler Development 3rd Edition
BB-12	*Bifidobacterium animalis subsp. lactis*
BERA	Brainstem Evoked Response Audiometry
BMI	Body mass index
CI	Confidence interval
DHA	Docosahexaenoic acid
DSMC	Data Safety Monitoring Board
EPA	Eicosapentaenoic acid
FAs	Fatty acids
HOME	The Home Observation for Measurement of the Environment
ICC	Intraclass Correlation Coefficient
IOWA	Infant Orienting with Attention
IPCW	Inverse Probability of Censoring Weighting
ITT	Intention-To-Treat
LGG	*Lactobacillus rhamnosus* GG
LMICs	Low- and middle-income countries
MRI	Magnetic Resonance Imaging
SAE	Serious Adverse Event
SCFAs	Short-chain fatty acids
SD	Standard deviation
SP	Study Physician
VPC	Visual Paired Comparison

1. ADMINISTRATIVE INFORMATION

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1.1 Document Version History

Version Date	Version	Author	Signature	Change Description	Reason/Comment
08/18/2025	1.0	RA		Initial release.	Not applicable.

1.2 Approvals

The undersigned have reviewed this plan and approve it as final. They find it to be consistent with the requirements of the protocol as it applies to their respective areas. They also find it to be compliant with ICH-E9 principles and confirm that this analysis plan was developed in a completely blinded manner (i.e. without knowledge of the effect of the intervention being assessed)

Name	Role on Study	Affiliation	Signature	Date
Rina Agustina	Principal investigator	Department of Nutrition, Faculty of Medicine, Universitas Indonesia–Dr. Cipto Mangunkusumo General Hospital; Human Nutrition Research Center, Indonesian Medical Education and Research Institute (HNRC-IMERI), Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia		2025-08-18
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2. STUDY SYNOPSIS

Interventions are urgently needed to enhance early-life brain and cognitive development, especially in low- and middle-income countries (LMICs) where multiple biomedical and psychosocial risks prevail. It is estimated that 36.8% of young children in LMICs perform poorly in at least one developmental domain,[1] Failure during this critical window of opportunity affects well-being into adulthood.[2] While nurturing care and stimulation are known to enhance early-life brain development, the multiplicity of factors that influence brain development from conception onward suggests additional interventions may remain unknown.

The gut-brain axis has emerged as a promising avenue for potentially enhancing brain development.[3] In infants, postnatal brain development parallels the maturation of gut microbiota, which suggests a potential role in development.[4] Moreover, supplementation of pregnant women with probiotics has demonstrated long-term benefits to infants[5], including reduced infant allergies, which could be mediated by regulatory T cells that may also affect brain development.[6,7] As such, supplementation during the prenatal period may provide other significant benefits. Other dietary components may enhance microbiome, such as omega-3 fatty acids (FAs). Indeed, both probiotics and omega-3 fatty acids are among the most studied dietary interventions regarding the gut microbiota, its response to challenge, the influence of the local immune cells, and the regulation of the short-chain fatty acids (SCFA) production.[8,9]

The maternal gut microbiota may affect fetal neurodevelopment through transplacental transport of microbiota-derived metabolites like SCFA and compounds like lipopolysaccharides.[10,11] Microbial metabolites and compounds can travel from the intestinal lumen to the bloodstream and the fetus via the placenta, providing biochemical signals that influence in-utero fetal neurodevelopment. A recent study in mice identified a connection between the maternal gut and fetal brain development through embryonic SCFA receptors, where SCFA from maternal gut microbiota were sensed by GPR41 and GPR43 in the sympathetic nerve of the embryo.[12] Although evidence is still limited, a systematic review of probiotic interventions on cognitive development identified one randomized trial in which *Lactobacillus rhamnosus* GG was given to the mothers for 4 weeks before delivery and continued for six months postpartum either to the mother (if breastfeeding) or to the child (if not).[13,14] The study showed a lower incidence of ADHD and Asperger syndrome at 13 years of age in the intervention group. These findings suggest that microbes and their metabolites in the maternal gut can affect fetal brain development and function in utero.

Both probiotics and omega-3 FAs could improve maternal gut microbiota diversity and demonstrate anti-inflammatory effects. Omega-3 FAs potentially synergize with probiotics to improve maternal microbiota composition by increasing SCFA production and supporting the growth of LPS-suppressing bacteria, such as *Bifidobacteria*.[15] Thus, a combined supplementation may be more effective than either intervention alone. Along with their influence on the intestine, maternal probiotics and omega-3 FAs supplementation may increase breast milk DHA levels and establish a healthier microbiota in breast milk[16,17], thereby supporting infant postnatal brain development.

While there is some evidence that maternal probiotics and omega-3 FAs supplementation could support early-life brain development, few trials have explored the effects of these interventions, especially their combined impact.[16-18] Previous research may not have adequately evaluated the effects of probiotic strains on neurodevelopment and may have overlooked socioenvironmental factors that could confound the results.[19-21] Moreover, studies on maternal supplementation during pregnancy have not assessed the effect on fetal brain development, which is necessary to better understand the connection between prenatal and postnatal outcomes. The Brain Probiotic and LC-PUFA Intervention for Optimum Early Life (BRAVE) is a double-blind randomized controlled trial in Jakarta, Indonesia, aiming to evaluate the impact and safety of supplementation of healthy pregnant women with the combination of probiotics and omega-3 FAs from the second trimester

up to 6 months postpartum on fetal brain growth and infant development at the age of 4 and 6 months. This statistical analysis plan outlines the methodology for analyzing fetal brain development and infant development.

2.1. Primary Objective

The overall objective of this analysis is to examine the effect of maternal probiotics and higher-dose omega-3 FAs supplementation, supported with government program supplements, healthy eating, and psychosocial stimulation, during pregnancy through 6 months postpartum on fetal brain growth assessed by magnetic resonance imaging (MRI) and infant development at the age of 4–6 months assessed using brainstem evoked response audiometry (BERA), Bayley Scales of Infant and Toddler Development 3rd Edition (Bayley-III), and eye-tracking assessments.

The hypotheses of the primary objectives are:

H1: Compared to the control group, fetus of women who receive daily probiotics and higher-dose omega-3 FAs supplements will show higher whole brain volume at 36–38 weeks of gestational age.

H2: Compared to the control group, fetus of women who receive daily probiotics and higher-dose omega-3 FAs supplements will show higher myelination index at 36–38 weeks of gestational age.

H3: Compared to the control group, children of women who receive daily probiotics and higher-dose omega-3 FAs supplements will show higher cognitive and cognitive composite scores of Bayley-III at the age of 4–6 months.

H4: Compared to the control group, children of women who receive daily probiotics and higher-dose omega-3 FAs supplements will show patterns of BERA indicators suggestive of better auditory function at the age of 4–6 months, specifically:

- Lower absolute latencies for wave I, wave III, and wave V
- Lower interpeak latencies for wave I–III interval, wave III–V interval, and wave I–V interval (central conduction time).

H5: Compared to the control group, children of women who receive daily probiotics and higher-dose omega-3 FAs supplements will show patterns of Infant Orienting with Attention (IOWA) task indicators suggestive of better attention at the age of 6 months, specifically:

- Lower mean latency
- Lower task error

H6: Compared to the control group, children of women who receive daily probiotics and higher-dose omega-3 FAs supplements will show patterns of Visual Paired Comparison (VPC) task indicators suggestive of better memory at the age of 6 months, specifically:

- Higher novelty preference score
- Lower mean familiarization fixation

2.2. Secondary Objectives

The hypotheses of secondary objectives are:

- Compared to the control group, fetus of women who receive daily probiotics and higher-dose omega-3 FAs supplements will show higher cerebrum, white matter, deep gray matter, cerebellum, and brainstem volumes at 36–38 weeks of gestational age.
- Compared to the control group, children of women who receive daily probiotics and higher-dose omega-3 FAs supplements will show higher receptive language, expressive language, language composite scores, fine motor, gross motor, and motor composite scores of Bayley-III at the age of 4–6 months.
- Compared to the control group, children of women who receive daily probiotics and higher-dose omega-3 FAs supplements will show higher cue facilitation and lower cue interference assessed by the IOWA task at the age of 4–6 months.
- The intervention effects on each outcome will differ by specific subgroups, including child sex, birth order, maternal pre-pregnancy body mass index (BMI) status, maternal anemia, maternal age, maternal education, socioeconomic status, and home environment.

2.3. Study Population

The Brave study was a parallel, individually randomized controlled trial (1:1 allocation ratio) that was carried out in Jakarta, Indonesia, from 2019 to 2022 by the Department of Nutrition, Faculty of Medicine, Universitas Indonesia–Dr. Cipto Mangunkusumo General Hospital and the Human Nutrition Research Center, Indonesian Medical Education and Research Institute (HNRC-IMERI), Faculty of Medicine, Universitas Indonesia. The primary objective of this study is to investigate how maternal probiotics combined with omega-3 FAs supported with government program supplements, healthy eating, and psychosocial stimulation could affect fetal brain development and later child brain functions and cognitive development at 4–6 months of age.

We screened potential participants through a patient listing from the maternal and child health unit in the local community health care and clinics. We recruited pregnant mothers in the first or second trimester of pregnancy at seven primary health centers in four municipalities in Jakarta.

Inclusion criteria of the trial:

- Indonesian pregnant women in the 2nd trimester of gestational period
- Healthy pregnancy (as measured by hemoglobin level, pregnancy status, pregnancy history)
- Having normal blood pressure
- Planning to stay in the study area until the child is 6 months old
- Willing to sign informed consent
- Having a legally acceptable representative who is capable of understanding the informed consent document and providing consent on the subject's behalf

Exclusion criteria of the trial:

- Having foreign objects in the body due to trauma, artificial heart valves, metal objects or ferromagnetic (plate, screw, clip, prosthetic), and electronic devices (pacemaker, cochlear implant, insulin pump), and being claustrophobic
- Having a history of previous gestational diabetes or having been diagnosed with gestational diabetes
- Having a history of type 1 and type 2 diabetes
- Severe anemia

2.4. Intervention

Provide a brief description of the details of each intervention being assessed including any potential control arm. If applicable, describe study drugs with dosages. Also include details of incomplete administration of study intervention e.g. acceptable reduction in study treatment for toxicity (where applicable).

Eligible pregnant mothers were randomly assigned to two groups (n=157 each):

(1) **Intervention group** consuming daily supplements of one capsule containing multistrain probiotics [Chr. Hansen A/S, Hørsholm, Denmark; *Lactobacillus rhamnosus* GG (LGG), *Bifidobacterium animalis subsp. lactis* (BB-12), *L. acidophilus* (LA-5)] and two capsules of higher-dose omega-3 FAs [Blackmores Pty Ltd, Australia; 720 mg eicosapentaenoic acid (EPA), 480 mg docosahexaenoic acid (DHA) in total], or

(2) **Control group** consuming one placebo capsule of probiotics (Chr. Hansen A/S, Hørsholm, Denmark) and two capsules of standard-dose omega-3 FAs (Blackmores Pty Ltd, Australia; 360 mg EPA, 240 mg DHA in total).

Both groups consumed the supplements from the 2nd trimester of pregnancy until 6-months post-partum, regardless of their breastfeeding status. Omega-3 FAs supplementation began at 22 weeks of pregnancy, while probiotics/placebo supplementation started at 26 weeks. The gestational age for all subjects was assessed using ultrasonography at the nearest community health center or Dr. Cipto Mangunkusumo General Hospital by trained general practitioners. These start times were chosen due to the late recognition of pregnancy in our population[23] and therefore to ensure uniform initiation for all participants. Additionally, probiotics/placebo supplementation commenced during the third trimester because of limited evidence regarding probiotics use in pregnancy before this period.[24,25] The placebo probiotics capsules were indistinguishable from the active capsules in appearance, taste, and odor. Likewise, the omega-3 capsules appeared identical across groups. All mothers in both groups also received psychosocial stimulation and healthy eating modules. These modules were delivered once during pregnancy (at 30–34 weeks of gestation) and twice during postpartum (between 0–1 month and between 1–2 months after birth). In the study sample, the majority of infants were breastfed, thus likely receiving DHA via their mother's breastmilk in addition to their prenatal exposure. Outcomes assessments were performed at 36–38 weeks of gestational age for fetal brain volume and when children reached the age of 4–6 months for Bayley-III, BERA, and eye-tracking assessments. All participants, outcome assessors, and field and research teams were blinded to the allocation of the intervention.

2.5. Randomisation and Blinding

Randomization was stratified by maternal educational status (≥9 years vs. <9 years of education) and municipality economic status (high economic areas in South and Central Jakarta vs. low economic areas in North and East Jakarta), using block sizes of 4 and 8. An independent, external third party performed the randomization prior to enrollment of the first participant and stored the allocation results in a sealed envelope. A second independent third party, working in a different department from the study team, also retained a copy of the randomization results. All study participants, field staff, and research team members were blinded throughout the trial. Only the two third parties holding the randomization results and the personnel responsible for labeling the treatments (who were not part of the field or research team) were unblinded.

2.6. Sample Size

Sample size calculations were based on the hypothesized effect size of 0.40 standard deviation (SD) between intervention and control groups on fetal whole brain volume at 36–38 weeks of

gestational age, and on Bayley-III cognitive scores, BERA indicators, and eye-tracking assessment indicators at 4–6 months of age (α=0.05 and 1-β=0.80). The original protocol assumed an effect size of 0.30 SD and a 20% attrition rate, resulting in a minimum sample size of 400 participants. However, due to recruitment challenges and increased loss to follow-up during the COVID-19 pandemic, which raised the anticipated attrition rate to 33.3% and constrained available funding, a protocol amendment was made. The target sample size was revised to detect a difference of 0.40 SD between groups, generally considered moderate and likely to be of clinical importance, resulting in a lowered estimated minimum sample size from 400 to 300 participants. Effect sizes between 0.30 and 0.40 SD were chosen based on prior findings: for Bayley-III domains, Cohen's d values in developmental cohorts and preterm interventions fall within this range; neuroimaging studies show between-group brain volume differences of approximately 0.30–0.50 SD in fetuses and neonates; and intervention-related physiologic/eye-tracking changes are typically described in similar effect size terms.[26–30]

2.7. Study Procedures

Mothers in intervention and control groups consumed the supplements from the 2nd trimester of pregnancy until 6 months post-partum, regardless of their breastfeeding status. Outcomes assessments were performed at 36–38 weeks of gestational age for fetal brain volume and when children reaching the age of 4–6 months for Bayley-III, BERA, and eye-tracking assessments. Fetal brain growth was assessed using MRI, and infant development was evaluated using the Bayley-III for cognitive scores and other developmental scores, BERA for auditory function, and eye-tracking assessments, which included the IOWA and VPC tasks for attention and memory indicators.

2.7.1 Fetal brain MRI

Pregnant women were scanned in a supine position for fetal brain volume assessment on a 1.5-Tesla General Electric MRI with an acquisition time of approximately 30 minutes, depending on fetal motion. We used multi-planar repeated T2-weighted half Fourier acquisition single-shot turbo spin echo (T2wHASTE) sequences performed with a 2 or 4 interleaved acquisition; effective echo time of 100 and 120 ms; repetition time of 1400–2000 ms; variable field of view based on fetal and maternal size; 2–3 mm slice thickness; no inter-slice gap; 256×204, 256×256, or 320×320 acquisition matrices; and in-plane resolution of 1 mm.

Two independent trained physicians manually segmented brain regions of interest (i.e., whole brain, cerebrum, white matter, deep grey matter, cerebellum, and brainstem) with the supervision of a radiologist specialized in neurodevelopment; any discrepancies were reconciled with the radiologist. The outcomes derived from this assessment include:

- Whole brain volume in cm^3 (continuous).
- Cerebrum volume in cm^3 (continuous).
- White matter volume in cm^3 (continuous).
- Deep grey matter volume in cm^3 (continuous).
- Cerebellum volume in cm^3 (continuous).
- Brainstem volume in cm^3 (continuous).

Due to data quality issues, the myelination index could not be quantified as intended and was therefore excluded from further analysis.

2.7.2 Bayley Scales of Infant and Toddler Development, 3rd edition (Bayley-III)

Two trained psychologists conducted the Bayley-III assessment in a room with minimal distractions. The subscales included in this analysis were cognitive, receptive language, expressive language, fine motor, and gross motor. Before administering the assessment to study participants, we evaluated inter-rater agreement between the two assessors using data from three children, which resulted in a 56% agreement rate—considered moderate. Following this result, a group discussion was held to address the areas of disagreement. Due to pandemic-related constraints and time limitations, we were unable to conduct additional inter-rater reliability assessments.

The Bayley-III assessments were conducted twice between 4 and 6 months of age. For this analysis, we will use the first assessment for each child as the outcome measure to avoid potential influence from prior exposure to the Bayley-III assessment within a short interval. The outcomes derived from this assessment include:

- Bayley-III *Cognitive score*: Z-score converted raw score of Bayley-III cognitive subscale (continuous).
- Bayley-III *Cognitive composite score*: Score based on conversion of Bayley-III cognitive scaled score (continuous).
- Bayley-III *Receptive language score*: Z-score converted raw score of Bayley-III receptive language subscale (continuous).
- Bayley-III *Expressive language score*: Z-score converted raw score of Bayley-III expressive language subscale (continuous).
- Bayley-III *Language composite score*: Score based on conversion of combined Bayley-III receptive and expressive communication scaled scores (continuous).
- Bayley-III *Fine motor score*: Z-score converted raw score of Bayley-III fine motor subscale (continuous).
- Bayley-III *Gross motor score*: Z-score converted raw score of Bayley-III gross motor subscale (continuous).
- Bayley-III *Motor composite score*: Score based on conversion of combined Bayley-III fine and gross motor scaled scores (continuous).

We will use Bayley-III raw scores generated from each subscale based on the number of items passed and convert them into a z-score.[31] Raw scores will also be converted into scaled scores based on normative data; the scale scores will be used to calculate cognitive, language and motor composite scores.[32]

2.7.3 Brainstem Evoked Response Audiometry (BERA)

BERA using a Cadwell 12-channel amplifier at 80 dB was performed when the children reached four months of age. Two independent, trained nurses conducted the assessments. Prior to conducting the assessments on study participants, we evaluated inter-rater reliability between the two assessors using the Intraclass Correlation Coefficient (ICC) in a sample of six children, which resulted an ICC of 0.99. The outcomes derived from this assessment include:

- Absolute latencies for wave I in ms (continuous).
- Absolute latencies for wave III in ms (continuous).
- Absolute latencies for wave V in ms (continuous).
- Interpeak latencies for wave I–III interval in ms (continuous).

- Interpeak latencies for wave III–V interval in ms (continuous).
- Interpeak latencies for wave I–V interval (central conduction time) in ms (continuous).

2.7.4 Eye-tracking assessments

Our eye-tracking assessments adopted a protocol by Prado et al (2020).[33] Eye-tracking-based IOWA and VPC tasks were performed when children reached the age of 6 months in a room with minimal distraction and quiet conditions by a trained psychologist. Children sat on their caregivers' laps, facing the screen. Caregivers were asked to look away from the monitor, close their eyes, or wear a blindfold. A monitor was positioned approximately 60 cm from the child's face. We monitored the caregiver and child using a webcam throughout the procedure. We used Tobii Pro X2–60 as the eye-tracking system to detect child's eye fixations when stimuli were presented on the monitor. The eye-tracking system recorded the coordinates (x, y) of the focal point of the infant's gaze at a rate of 60 Hz. Tobii I-VT Fixation Filter was used to classify fixations.

We will evaluate child attention using the IOWA task based on Ross-Sheehy (2015).[34] This task assesses visual attention based on the precue presented to the children. Each trial of the IOWA task consisted of the following:

1. Central fixation

Before every trial in IOWA task, a central fixation appeared as a bright yellow dynamic smiley face that loomed from small (0° 52' width X 0° 57' height) to large (4° 35' width X 5° 9' height) at a rate of approximately 1.5 Hz, accompanied with classical music. When the infant's gaze was fixed on the central fixation, the eye-tracking assessor pressed a key to advance to the trial.

2. A 100 ms spatial precue depending on the cue type

The trial period in the IOWA task involves several cue types: 3 experimental conditions (*valid, invalid*, and *double cues*) and 1 control condition (*no cue*). A black dot serves as a spatial precue preceding a randomly presented target image. The number and location of the presented black dot depend on the types of cue conditions.

3. A 100 ms blank screen
4. A target image displayed for 1000 ms

The target images consisted of 96 colorful everyday objects, some familiar and others unfamiliar to the children, such as fruits, balls, and baskets, on a gray background (RGB: 136, 136, 136).

In the *valid cue* condition, both the cue and target image appeared on the same side, while in the *invalid cue* condition, the target image was presented on the opposite side of the cue. The *double cue* condition involves cues on both sides, with the target image presented at the spatial location of 1 of the 2 cued locations. In the baseline condition, *no cue* is presented, but the target image still appears on either the left or right side of the screen. Each child was exposed to up to 24 trials per cue condition, with half of the target images on the screen's right side. No music or tone was presented during the trials.

The outcomes derived from this task will include:

- IOWA *Mean latency*: Average latency to the target image across all four spatial cues on correct trials (continuous).
- IOWA *Task error*: The degree of interference from the invalid spatial cue that was present in double and invalid cue condition = 1 – (mean(Percor_double, Percor_invalid)) (continuous).

- IOWA *Cue facilitation*: The degree of facilitation due to the valid cue compared to the no cue condition = (Mean_nocue - Mean_valid)/Mean_nocue (continuous).
- IOWA *Cue interference*: The degree of interference due to the invalid cue compared to the no cue condition = (Mean_invalid - Mean_nocue)/Mean_nocue (continuous).

We will create area of interests (AOIs) around the central fixation, left image, and right image. Based on this AOI, each IOWA trial was classified as correct if the child's first fixation fell on the target image AOI (either left or right) after the onset of this target image. If the trial is correct, we calculated the *mean latency*. We excluded any trials when children looked at the central fixation < 200 ms to ensure that the fixation was in response to the target and not started before the child attended to the target.[33,34] We also excluded correct trials with mean latency < 100 ms and > 1000 ms. A mean latency of 100 ms is considered too fast to reflect child's response to the target, and a mean latency of 1000 ms may indicate that the child was off-task. Task error will be determined by the proportion of correct in invalid and double cue trials.

We will use the VPC task to assess visual recognition memory. This task has been previously used in diverse contexts.[35] We will compare the proportion of time the child spends looking at a novel face compared to the previously seen face (i.e., novelty preference). VPC consists of four trials for four different pairs of Indonesian faces (i.e., adult male-adult female, adult male-child male, adult female-child female, and child male-child female). Each trial comprises a *familiarization* followed by a *recognition memory* period.

1. Familiarization period: Two culturally familiar faces were presented on the left and right sides of the screen.
2. Recognition memory period: A new pair of faces was presented, consisting of the same face shown in familiarization period (familiar stimulus) on one side and a novel face on the other side. These stimuli were side-reversed after the first 5 seconds.

The outcomes derived from this task will include:

- VPC *Novelty preference score*: Total time looking at the novel stimulus divided by the total time looking at stimulus during the recognition memory period (continuous).
- VPC *Mean familiarization fixation:* Mean fixation across all familiarization trials for which data are available (continuous).

We will create the AOIs for each face that was presented: 1 AOI covered the right side of the screen from the right edge of the central image to the right edge of the screen and from top to bottom, and 1 AOI covered the mirror image on the left side of the screen. We excluded trials with <1 s of looking time during either familiarization or recognition memory periods.

2.8. Deviations from Protocol

During COVID-19 pandemic, research activities were strictly adjusted with the COVID-19 prevention measures for both personnel and subjects. Some adjustments for the project field implementation were conducted during the outbreak, including (1) implementing routine COVID-19 screening, providing adequate PPE and education for the fieldworkers about the prevention measures, (2) supplement delivery and anthropometric assessment tools using online motorcycle taxi services with a protocol developed to ensure the safety of supplement bottle transfer from the research personnel to the motorcycle taxi driver, and from the driver to the mothers, including safe distancing and disinfection procedures, (3) educating and providing sanitation supplies to the mothers, (4) mothers might not attend MRI assessment and blood sample collection if not comfortable with visiting non-COVID-18 referral hospital and laboratory. Several implementation protocols—Plan B (social distancing), Plan C (social distancing and remote working), and Plan D

(intensive social distancing and remote working)—were developed and adapted to the evolving pandemic dynamics. These protocols were based on guidance from the Indonesian Ministry of Health, permits from local government and ethics committees, the Data Safety Monitoring Board, and the FDA's Guidance on Conduct of Clinical Trials of Medical Products during the COVID-19 Pandemic.

Full details of the background to the trial and its design are presented in the protocol.

3. GENERAL STATISTICAL METHODOLOGY

3.1. Objectives of Analysis Plan

This analysis plan covers all primary objectives and selected secondary objectives related to fetal brain and infant development assessments.

3.2. Analysis Software

R version 4.3.2 – primarily used for analyses and results preparation

Stata version 14.1 – primarily used for data cleaning and management

3.3. Data verification

All data underwent verification, consistency, and range checks prior to analysis. Field supervisors reviewed data entries from enumerators against the source documents to ensure accuracy. Extreme outcome values and range checks were performed for age at assessment across all outcomes, including fetal brain assessment, Bayley-III scores, BERA results, and indicators of eye-tracking assessment. All data reviews and cleaning procedures were performed while the investigators were blinded to treatment allocation.

3.4. Definition of Baseline

Baseline assessments were performed between 18–22 weeks of gestation to collect data on potential covariates, including sociodemographic characteristics, anthropometric measurements, and hemoglobin assessment.

3.5. Definition of analysis populations

For all outcomes, we will perform complete-case analysis, which include all subjects with outcome data regardless their compliance to the intervention.

3.6. Definitions related to Adverse Events (AEs)

- *Adverse Event* means any undesirable clinical experience, including clinically significant laboratory values, occurring to the subject during the study, whether or not considered related to the investigational product or the study procedures. This includes exacerbations of pre-existing conditions.
- *Adverse Effect*: When an Adverse Event has been determined to be related to the test product or the study procedures, it is considered an Adverse Effect.
- *Exacerbations of Pre-existing Conditions* means a condition that developed before the study but which worsened or occurred with increasing frequency as study progressed.
- *"Unexpected" Adverse Events* are defined as those that would not be expected among subjects who are supplemented with the test product at safe levels.
- *Serious Adverse Event* (SAE) means any event that results in death, is immediately life threatening requires inpatient hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect.
- *Life-Threatening* means the subject is at immediate risk of death from the event as it occurred.

The intensity of the Adverse Event was rated as mild, moderate, or severe using the following criteria:

- Mild events require minimal or no treatment and do not interfere with the subject's daily activities.
- Moderate events result in a low level of inconvenience or concern for the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe events interrupt a subject's usual daily activity and typically require systemic drug therapy or other treatment. Severe events are usually incapacitating.

If any, changes in the intensity of an AE were documented to allow an assessment of the duration of the event to be performed at each level of intensity.

3.6.1 Reporting of Adverse Events

All AEs reported or observed during the study were recorded in the Adverse Event Report Form/Case Report Form by the study physician (SP) from the study team and the SP from the community health center assigned to the relevant subdistrict. The need to capture this information was not dependent on whether the AE was associated with the test products or the study procedures. The SP at the community health center was responsible for determining the relationship of each AE to the test products or study procedures, assessing its intensity, and following all AEs until they are adequately resolved. Only the SP at the community health center or the referred health center was authorized to establish diagnoses and provide appropriate treatment. Any actions taken in response to an AE, along with the subsequent outcomes, were also recorded. All AEs were classified using ICD-10 codes, with preeclampsia cases recorded separately.

The Data Safety Monitoring Board (DSMB) evaluated and discussed the accumulated adverse events to monitor participant safety and provide recommendations on whether to continue, modify, or terminate the trial for certain participants. This review occurred twice for each study area (South, Central, North, and East Jakarta) during the intervention period. If necessary, in the worst-case scenario, the DSMB could decide to stop the trial. An SP coordinator from the study team was assigned to ensure all of these procedures were carried out appropriately.

3.6.2 Reporting of Serious or Unexpected Adverse Events

If, according to the review of the study physician, an adverse event met the criteria of a SAE or an unexpected adverse event, the following procedure was followed:

- The Principal Investigator reported the SAE or unexpected AE with the potential causality towards participation in the study, immediately (and in all cases within 24 hours) of becoming aware of the event to the DSMB.
- The Principal Investigator provided the minimal information i.e. date of birth, subject's number, date of SAE or unexpected AE, serious adverse event term and causality, immediate follow up.
- The DSMB had to file a report, including the duration and actions taken, and provided advice whether or not the event is considered attributable to the test product or the study procedures and whether the study should be continued or modified. This report was delivered to the Principal Investigator as soon as possible to take further action. The Principal Investigator informed the Ethical Committee of the Faculty of Medicine, Universitas Indonesia, and Dr. Cipto Mangunkusumo National Hospital within three calendar days.

- The PI will inform the grantor of the project and providers of intervention about the SAE or unexpected AE, action taken, and recommendation of DSMB.
- The subject who experienced SAE or unexpected AE was followed and treated clinically and by laboratory studies until all parameters had returned to normal or have resolved.

3.7. Adjustment for Multiplicity

No adjustment for multiple comparisons will be performed.

3.8. Interim Analyses

We assessed blinded data every week to review and resolve errors. We monitored adherence to supplementation throughout the trial and responded to any issues that resulted in low adherence.

DSMB monitored data for evidence of any AEs, SAEs, or deaths related to the allocation, and provided recommendations if need to stop the trial prematurely based on predefined protocol. Every SAE was reported to the DSMB within 24 hours to provide recommendation of continuation, modification, or termination. AEs and SAEs were monitored daily by study site physician team, and a summary report were shared monthly to the DSMB. The DSMB met twice for each municipality (South, Central, North, and East Jakarta) throughout the study to review the compiled data. No interim analyses were planned for this study.

3.9. Handling of Missing Data

We will handle missing data first by tabulating the proportion of subjects lost to follow-up by allocation groups and examining if missingness differs significantly (e.g., >10% absolute difference) by allocation groups or key baseline characteristics. Additionally, baseline characteristics of participants with available outcome data will be compared to those with missing outcome data. To further assess potential bias introduced by missingness, we will evaluate balance in baseline covariates between allocation groups, both in the original trial sample and the sample retained at follow-up. Efficacy analyses and other exploratory analyses will be performed in the full analysis set (i.e., all subjects randomly assigned to the study, including those who did not receive a dose of study treatment).

3.10. Definitions Related to Estimands

We will use treatment policy estimand with an intention-to-treat analysis for all primary and secondary objectives. The treatment policy estimand evaluates the treatment effect on the variable regardless of the intercurrent event. In other words, the outcome of interest is considered whether or not an intercurrent event occurred before its measurement, e.g. the final outcome is of interest irrespective of whether the participant takes additional medication or has low compliance.

4. DESCRIPTIVE STATISTICS

4.1. Recruitment and Follow-up

The status of participants will be reported using a CONSORT flowchart. Caregivers were informed that the children could withdraw from the study at any time. A senior staff person visited caregivers who indicated that the children wanted to withdraw to discuss the family's concerns and reasons for withdrawal. Children who had already withdrawn their participation were not followed up on unless they requested to rejoin the trial. All procedures related to recruitment, follow-up, and withdrawal were conducted in accordance with the trial's ethical approvals.

4.2. Baseline Characteristics

We will present characteristics of the study sample with continuous data as mean and SD or as median with 25th and 75th percentiles. Categorical variables will be presented as frequencies and percentages. We will indicate sample sizes for variables with more than 20% missing observations in the footnotes. Variables of the baseline characteristics are listed in **Table 1**, which best describe the study population, particularly in relation to child nutrition and cognition, as our predictor and outcome of interest in this analysis.

Table 1. Characteristics of the study sample

Variable	Definition
Baseline	
Child sex	2 categories: Female or male
Birth order	2 categories: First born or ≥ second-born
Municipality economic status	2 categories: High (Central and South Jakarta) or low (North and East Jakarta)
Maternal height	Maternal height in centimeters
Maternal pre-pregnancy BMI	BMI in kg/m^2
Maternal hemoglobin level at baseline	Hemoglobin level in g/L
Maternal history of hypertension	2 categories: With a history of hypertension or without any history of hypertension
Maternal age	Maternal age in years
Maternal education	3 categories: No education/incomplete primary/unknown, Completed primary, Completed secondary or greater
Number of children	Number of children under 5 years old in the household
Number of household members	Total number of people in the household
Maternal marital status	3 categories: Monogamous marriage, polygamous marriage, or unmarried [single, divorced, widowed]
Maternal occupation	2 categories: Housewife or working mother
Paternal occupation	2 categories: Non-private sector and unemployed, or private sector
Monthly household expenditure	Household expenditure in Indonesian Rupiah per month
Endline	
Child age at outcome assessment	Child age in months (or gestational week for fetal brain outcome)
HOME inventory	HOME inventory score at the age of 4–6 months

Screen time	Screen time per day in minutes at the age of 4–6 months

HOME inventory, the Home Observation for Measurement of the Environment inventory.

4.3. Protocol Deviations

Any protocol deviations were reported to the Health Research Ethics Committee - Faculty of Medicine Universitas Indonesia and Cipto Mangunkusumo Hospital (HREC-FMUI/CMH).

4.4. Compliance

We will report the adherence to the supplementation for both groups based on the remaining supplements returned by the participants every month and verified through a monthly compliance record filled out by both subjects and field workers.

4.5. Concomitant Therapies

Concomitant interventions, including the use of other medicines, were documented using compliance records completed by both participants and field workers, capturing the type, duration, and frequency of use. These records were reviewed and summarized by the SP coordinator and reported to the DSMB.

5. ANALYSIS OF THE PRIMARY OUTCOME(S)

5.1. Main Analysis

We will perform all analyses after the last participant in the follow-up study has completed their visit. We will review the characteristics of the study sample as blinded cohort. First, we will use fake IDs and scrambled group code to write the script for analysis and create dummy tables and figures. Once the script, tables, and figures are ready, we will insert the correct IDs with blinded group code (e.g., A vs. B) and create the draft of the interpretation of the results for each case (e.g., if "A" is the Control group vs. if "A" is the Intervention group). We will finally reveal the group assignments after all authors have reviewed and approved the results for the primary outcomes. Unblinded analyses will be performed for secondary analyses, such as effect modification and subgroup analyses.

For all outcomes, the primary analysis to answer each objective will be a minimally adjusted model controlling only for child age at assessment and the randomization stratification factors (maternal education and municipality economic status). In secondary analyses, we will estimate adjusted parameters by including additional covariates—baseline characteristics, as well as post-enrollment and follow-up variables—in addition to those included in the primary analyses.

Unless otherwise specified, we will use two-sided confidence intervals at the 0.05 significance level for all analyses. For example, we are not powered to conduct subgroup/effect-modification analyses, so these secondary objectives will be exploratory and considered significant at the α=0.10 level. All hypothesis testing in this analysis plan is based on a two-sided superiority framework.

5.1.1 Minimally adjusted analysis

We will evaluate the intention-to-treat (ITT) effects of the complete cases between allocation groups on each outcome. Our null hypothesis is that the estimated mean differences in the outcome do not differ significantly between the allocation groups. The null hypothesis will be rejected if the estimated mean differences are significantly different between the allocation groups.

We will fit linear regressions to assess the intervention's effects on fetal brain growth and infant development, controlling for *Allocation Group*, *Child Age at outcome assessment, Maternal Education,* and *Municipality Economic Status* as fixed effects. We will first check the normality and homoscedasticity of residuals using the Shapiro-Wilk and Breusch-Pagan tests. If any model assumptions (e.g., linearity, constant variance, data heaping) are violated, we will explore data transformations before analysis (e.g., log transformation, square root transformation). If no suitable transformations can be made, the outcomes will be analyzed using ranked data or as an ordinal categorical variable in cases of extreme data heaping. We will also identify outliers using Cooks' D, histograms, and scatter plots of the variables. Outliers that are possible will be kept, and sensitivity analyses will be completed with and without their inclusion. Outliers that are impossible (e.g. VPC Novelty Preference Score greater than one, IOWA mean latency less than zero, etc) will be corrected if possible or recorded to missing.

After fitting the models, we will estimate the mean differences of the variables of interest (e.g., *Allocation Group*) and conduct pairwise comparisons. We will not specifically evaluate model fit because it is not our primary interest.

5.1.2 Baseline and fully adjusted analyses

We will also perform baseline and fully adjusted analyses with covariates for each outcome. First, we will perform bivariate analyses using the likelihood ratio test to test the association between the covariates with each outcome. Covariates with a p-value < 0.1 will be included in the adjusted analysis. Covariates with little variation in the study population (e.g., prevalence < 5%) will be

excluded. We will also evaluate the collinearity between potential covariates using the Variance Inflation Factor.

Potential covariates are listed below:

- Child sex: female or male
- Birth order: first-born or ≥ second-born
- Municipality economic status: High or low
- Maternal height in centimeters
- Maternal pre-pregnancy BMI: BMI in kg/m^2
- Maternal hemoglobin level at baseline in g/L
- Maternal history of hypertension at baseline: With or without any history of hypertension
- Maternal age in years
- Maternal education: no education/incomplete primary/unknown, completed primary (6 years of formal education), completed secondary (9 years of formal education), completed greater than secondary
- Number of children under 5 yo in the household
- Number of household members
- Maternal marital status: monogamous marriage, polygamous marriage, or unmarried [single, divorced, widowed]
- Maternal occupation: housewife or working mother
- Paternal occupation: non-private sector and unemployed or private sector
- Monthly household expenditure in Indonesian Rupiah per month
- HOME inventory score (home environment)
- Screen time per day in minutes
- Staff members performing the assessment (for Bayley-III)

Two versions of adjusted analyses will be conducted as described below.

- Baseline adjusted model: This model will include covariates in minimally adjusted model and any of the following baseline covariates that are significantly associated at $p < 0.1$ with the outcomes: child sex, birth order, maternal height, maternal pre-pregnancy BMI, maternal hemoglobin, maternal age, paternal age, paternal education, number of children under 5 yo in the household, number of household members, maternal marital status, maternal occupation, paternal occupation, household expenditure.
- Fully adjusted model: Then, we will adjust for any covariates collected at follow-up (HOME inventory score, screen time) that are significantly associated at $p < 0.1$ with the outcomes. For any covariates collected after baseline, we will check whether any differences are based on allocation groups at $p < 0.1$. Significant differences indicate potential mediators. Therefore, we will exclude them from the model.

Table 2. Primary Estimands

Primary Estimand for Primary Analysis					
Population	**Treatment**	**Outcome**	**Summary Measure**	**Potential intercurrent events**	**Strategy for intercurrent event**
ITT	Initially randomised treatment (Intervention v. Control) and any subsequent changes to treatment over treatment period	Whole fetal brain volume in cm^3 at 36–38 weeks of gestational age (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in whole brain volume in cm^3, adjusted for gestational age at assessment and the randomization stratification factors (maternal education and municipality economic status)	1. Treatment discontinuation without study withdrawal 2. Early study withdrawal 3. Treatment non-compliance (intermittent or partial treatment adherence not resulting in treatment discontinuation) 4. Dose adjustment	Treatment Policy for all intercurrent events - all observed values will be used, regardless of whether or not the subject had experienced the intercurrent event
		Bayley-III Cognitive score at 4–6 months: Z-score converted raw score of Bayley-III cognitive subscale (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in Bayley-III cognitive score, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		Bayley-III Cognitive composite score at 4–6 months: Score based on conversion of Bayley-III cognitive scaled score (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in Bayley-III cognitive composite score, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		BERA absolute latencies at 4–6 months for wave I in ms (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in absolute latencies for wave I, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		BERA absolute latencies at 4–6 months for wave III in ms (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in absolute latencies for wave III, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		BERA absolute latencies at 4–6 months for wave V in ms (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in absolute latencies for wave V, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		BERA interpeak latencies at 4–6 months for wave I–III interval in ms (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in interpeak latencies for wave I–III, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		BERA interpeak latencies at 4–6 months for wave III–V interval in ms (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in interpeak latencies for wave III–V, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		BERA interpeak latencies at 4–6 months for wave I–V interval (central conduction time) in ms (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in interpeak latencies for wave I–V, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		

		IOWA mean latency at 6 months	Minimally adjusted mean difference (95%CI) between treatment arms in IOWA mean latency, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		IOWA task error at 6 months	Minimally adjusted mean difference (95%CI) between treatment arms in IOWA task error, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		VPC novelty preference score at 6 months	Minimally adjusted mean difference (95%CI) between treatment arms in VPC novelty preference score, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		VPC mean familiarization fixation at months	Minimally adjusted mean difference (95%CI) between treatment arms in VPC mean familiarization fixation, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		

5.2. Sensitivity Analyses

In all primary analyses for the primary outcomes, inverse probability of censoring weights (IPCW) will be estimated to address potential bias from outcome attrition.[36] The missingness model will be fit using logistic regression with a prespecified set of covariates, including allocation group, randomization stratification factors (maternal education and municipality economic status), baseline characteristics listed in **Table 1**, and other primary outcomes measured prior to the outcome visit (e.g., fetal whole brain volume when modeling the cognitive score) that are plausible predictors of both censoring and the outcome. Stabilized weights will be computed conditional on allocation group and the randomization stratification factors, and weights will be truncated at the 1st and 99th percentiles (0.5th and 99.5th in sensitivity analyses). Diagnostics will include summaries of the weight distribution, effective sample size, and covariate balance before and after weighting. Robustness to model misspecification will be assessed using augmented IPCW estimators.

We will not perform a per-protocol analysis given the high compliance with both the probiotics/placebo and omega-3 FAs interventions.

5.3. Subgroup Analyses

As a secondary objective, we will assess for potential effect modification on the primary outcomes by testing an interaction term between the allocation group and the factors listed below. We will present the differences between the Intervention and Control groups stratified by the effect modifier if the interactions are significant at $p<0.1$.

- **Child sex:** Biological differences, different care practices, or other psychosocial practices may modify the effect of interventions on girls vs boys.
- **Birth order**: Compared to second-born or greater (multiparous mothers), first-born tend to be smaller and may be associated with lower development, thus modifying the effect of interventions.

- **Maternal pre-pregnancy BMI status:** Overweight/obesity may change gut microbiota, including during pregnancy; therefore, it may modify the effect of our intervention between mothers with vs without overweight/obesity using a BMI cutoff of ≥25 kg/m^2.
- **Baseline maternal anemia:** Anemia during pregnancy has been associated with slower brain development, which may modify the effect of interventions between mothers with vs without anemia.
- **Baseline maternal age:** Biological differences, differential care practices, or differential social support networks due to different maternal age may modify the effect of the interventions on child development. We will first use maternal age as a continuous variable to test interactions. Significant interactions will be further examined with stratified analyses.
- **Baseline maternal education:** Maternal education has been consistently associated with child development and may modify the effect of interventions between mothers with low vs high education. We will use a cutoff of incomplete secondary schooling (<9 years of formal education) to define “low education” and secondary completion or greater as “high education”.
- **Baseline socioeconomic status:** We will categorize subjects based on monthly household expenditure into quintiles, then dichotomize into the highest quintile vs. the lowest four quintiles.
- **HOME Inventory score at 4–6 months**: Children from households with lower nurturing and stimulation of the home environment may have greater potential to benefit from the intervention. We will first use the HOME Inventory score as a continuous variable to test interactions. Significant interactions will be further examined with stratified analyses.

SECONDARY OUTCOMES

5.1 Main Analysis

For all secondary outcomes, we will apply the same analytical approach used for the primary outcomes, including both the primary and secondary analyses. The primary analysis will use a minimally adjusted model, controlling only child age at assessment and the randomization stratification factors (maternal education and municipality economic status). In the secondary analyses, we will estimate adjusted parameters by adding additional covariates—baseline characteristics as well as post-enrollment and follow-up variables—in addition to those included in the primary analysis.

Table 3. Secondary Estimands

Secondary Estimand for Primary Analysis					
Population	**Treatment**	**Outcome**	**Summary Measure**	**Potential intercurrent events**	**Strategy for intercurrent event**
ITT	Initially randomised treatment (Intervention v. Control) and any subsequent changes to treatment over treatment period	Whole fetal brain volume in cm^3 at 36–38 weeks of gestational age (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in whole brain volume in cm^3, adjusted for gestational age at assessment and the randomization stratification factors (maternal education and municipality economic status)	5. Treatment discontinuation without study withdrawal 6. Early study withdrawal 7. Treatment non-compliance (intermittent or partial treatment adherence not resulting in treatment discontinuation) 8. Dose adjustment	Treatment Policy for all intercurrent events - all observed values will be used, regardless of whether or not the subject had experienced the intercurrent event
		Cerebrum volume in cm^3 at 36–38 weeks of gestational age (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in cerebrum volume in cm^3, adjusted for gestational age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		Deep grey matter volume in cm^3 at 36–38 weeks of gestational age (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in deep grey matter volume in cm^3, adjusted for gestational age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		Cerebellum volume in cm^3 at 36–38 weeks of gestational age (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in cerebellum volume in cm^3, adjusted for gestational age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		Brainstem volume in cm^3 at 36–38 weeks of gestational age (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in brainstem volume in cm^3, adjusted for gestational age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		Bayley-III Receptive language score at 4–6 months: Z-score converted raw score of Bayley-III receptive language subscale (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in Bayley-III cognitive score, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		

		Bayley-III *Expressive language score*: Z-score converted raw score of Bayley-III expressive language subscale (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in Bayley-III expressive language score, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		Bayley-III *Language composite score*: Score based on conversion of combined Bayley-III receptive and expressive communication scaled scores (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in Bayley-III language composite score, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		Bayley-III *Fine motor score*: Z-score converted raw score of Bayley-III fine motor subscale (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in Bayley-III fine motor score, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		Bayley-III *Gross motor score*: Z-score converted raw score of Bayley-III gross motor subscale (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in Bayley-III gross motor score, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		Bayley-III *Motor composite score*: Score based on conversion of combined Bayley-III fine and gross motor scaled scores (continuous)	Minimally adjusted mean difference (95%CI) between treatment arms in Bayley-III motor composite score, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		IOWA cue facilitation at 6 months	Minimally adjusted mean difference (95%CI) between treatment arms in IOWA cue facilitation, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		
		IOWA cue competition at 6 months	Minimally adjusted mean difference (95%CI) between treatment arms in IOWA cue competition, adjusted for child age at assessment and the randomization stratification factors (maternal education and municipality economic status)		

5.2 Sensitivity Analyses

We will not perform sensitivity analyses for secondary outcomes.

5.3 Subgroup Analyses

We will not perform subgroup analyses for secondary outcomes.

6. SAFETY OUTCOMES

AEs and SAEs, including participant deaths and hospitalizations, were monitored by study site physicians and reported to the DSMB in accordance with the DSMB protocol. Safety analyses will be conducted in the safety analysis set, defined as all participants who received at least one dose of the study treatment. Safety data, including laboratory parameters, will be summarized descriptively by treatment group, reporting the number and percentage of participants experiencing each AE or SAE.

7. TABLES AND FIGURES

7.1. List of Tables

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