Does antenatal supplementation with omega-3 affect child development and behavior during the first six months of life? A randomized double-blind placebo-controlled trial

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Research Article

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Abstract

Purpose

This randomized double-blind placebo-controlled trial aimed to evaluate the effect of antenatal omega-3 supplementation on child development and behavior during the first six months of life.

Methods

Participants with gestational age between 22–24 weeks were randomized in placebo (olive oil; n = 30) or omega-3 (fish oil; n = 30) groups and supplemented until childbirth. Fish oil capsules provided 1440mg/day of DHA. Child development was assessed using the Survey of Well-being of Young Children in the first, fourth and sixth month of life. Intention-to-treat and per-protocol analyses were performed using Generalized Estimating Equations.

Results

The comparison between groups showed no differences in the Developmental Milestones score at any time, but when compared to the first month, the omega-3 group showed an increase at the fourth and sixth month. Such increase was not observed in the placebo group. No differences were found between groups for Irritability and Inflexibility scores, however, higher scores for Difficulty with Routine were observed in the placebo group when compared to omega-3 at first, fourth and sixth month.

Conclusion

There were no differences between groups for child development, but the omega-3 group showed an increase in this score over time. The placebo group had greater difficulty with routine than the omega-3 group, indicating a beneficial effect of antenatal supplementation on child behavior.

Trial registration:

ReBec U1111-1215-7952 (June 16th 2018).

What Is Known

- Omega-3 fatty acids, especially docosahexaenoic acid (DHA), are found in different cell membranes, but more concentrated in the brain, playing an important role in child's behavior and development.
- DHA deficiency in pregnant women may limit neurodevelopment of term infants and dietary intakes are often insufficient, therefore, antenatal supplementation may be necessary to achieve daily recommendation.

What Is New

- The omega-3 group had lower difficulty with routine than the placebo group, indicating a beneficial effect of antenatal supplementation on child behavior.

- The difficulty with routine subscale comprises mostly questions about infant sleep, and DHA supply during the intrauterine period may modify brain phospholipids, resulting in a more mature central nervous system, which is essential for a healthy sleep pattern.

Introduction

The gestational period is a window of opportunity to promote child adequate growth and development [1]. During this period, several nutrients are transported to the fetus, therefore, the maternal nutrient intake is important to ensure the adequate supply for their child [2].

Omega-3 fatty acids are one of the nutrients that increase plasma concentrations during pregnancy, because of an enhanced mobilization of docosahexaenoic acid (DHA) from the maternal adipose tissue to the placenta [3, 4]. This nutrient is found in different cell membranes, but is concentrated in the brain, playing an important role in central nervous system development [5]. The accumulation of DHA in the brain tissue begins during the intrauterine period, around the second pregnancy trimester, when neurodevelopment accelerates [4, 5].

Although the DHA have been widely associated to child visual, cognitive, and motor development, previous studies showed mixed results [6–9]. A systematic review reported seven clinical trials that evaluated the effect of omega-3 supplementation during pregnancy on cognition, attention, behavior, language and motor development, however, only one trial showed a positive effect, concluding that further investigations are needed [10].

On the other hand, a previous study was able to demonstrate that DHA deficiency in pregnant women limited neurodevelopment of term infants [8], highlighting the importance of adequate omega-3 intake during this period. Also, the need to provide adequate amounts of DHA during pregnancy has become a great concern worldwide, since dietary intakes are insufficient in different populations [11], therefore, antenatal supplementation may be necessary to achieve daily recommendation.

Considering the scenario described above, the present trial aimed to evaluate the effect of antenatal omega-3 supplementation on child development and behavior during the first six months of life.

Materials And Methods

Design and participants

This randomized, double-blind, placebo-controlled trial was conducted with data from the main study entitled "*Omega-3 supplementation during pregnancy to prevent depressive symptoms and possible effect on breastfeeding, child growth and development*", designed according to CONSORT 2010 recommendations [12].

The study was approved by the Research Ethics Committee under number 87705018.0.0000.5149 and registered by the Brazilian Registry of Clinical Trials (ReBec) under number U1111-1215-7952 (http://www.ensaiosclinicos.gov.br/rg / RBR-6gbzw6/). All participants signed a consent form after receiving the necessary information and expressed spontaneous interest to participate in the trial.

Low-risk pregnant women attending antenatal care at a public ambulatory in Brazil were recruited. Inclusion criteria were age between 20–40 years old and gestational age between 22–24 weeks. All cases diagnosed by the medical team as high-risk pregnancy, and who reported supplementation with fish oil in the previous month or

intolerance to fish/seafood were not included. Women who were under psychiatric treatment or diagnosed with depression were also not included in the sample since they served as exclusion criteria for the main study.

Pregnant women were randomized at a proportion of 1:1 in placebo and omega-3 groups using the MATLAB® software. The omega-3 group received fish oil capsules, while the placebo group received olive oil capsules with equivalent caloric content. Both omega-3 and placebo capsules had 1000mg content and were approved according to microbiological safety and nutritional composition. All participants received detailed guidance on how to use and storage the supplement and were instructed to take two capsules a day until delivery (≈ 16 weeks) and no dietary changes were prescribed. The supplementation with two capsules of fish oil a day offered 260mg of EPA (eicosapentaenoic acid) and 1440mg of DHA (docosahexaenoic acid).

Women were evaluated at baseline (22–24 gestational weeks) and the mother-child dyad were evaluated at two weeks, one month, four months, and sixth months postpartum (Fig. 1).

Data collection

Sociodemographic data, pregnancy information and food consumption were evaluated. At baseline, women were evaluated in person and after delivery via telephone interviews.

Sociodemographic data included maternal age, education, marital status, occupation, parity, and per capita income. Gestational age was calculated based on the first ultrasound and participants were also asked about pregnancy intention.

Food consumption was assessed using two 24 hours recall (24hR), applied on non-consecutive days, with a maximum interval of one week between them. Omega-3 composition was computed using Brasil Nutri® software [13].

Women received a new bottle of supplement capsules once a month during pregnancy, when they were asked whether they had continued supplementation in the previous month and whether there was any intolerance or discomfort. To estimate compliance, the participants referred the number of days not supplemented. Women who discontinued supplementation, or who presented compliance below 80% [14], were excluded from the analyses.

After delivery, women and their respective children were assessed by telephone interview for childbirth information, breastfeeding practice, and child development.

Child nutritional status was assessed using weight, length and head circumference at birth collected from medical records, and classified according to gestational age using the *INTERGROWTH-21st* curves [15].

Regarding breastfeeding, the participants were asked about breastfeeding in the first hour of life, whether they are currently breastfeeding, and whether they are exclusively breastfeeding and breastfeeding on demand.

To assess child development, the Survey of Well-being of Young Children (SWYC) questionnaire was applied in the first, fourth and sixth month of life. For preterm children, the corrected age was adopted. The SWYC is a questionnaire directed for parents of children aged one to 65 months, proposed by Perrin et al. [16], translated and validated for the Brazilian population by Moreira et al. [17]. The questionnaire is divided into three domains: developmental, emotional/behavioral, and family context, and was considered suitable for application via telephone interview [17]. The developmental domain is a checklist containing 10 questions about child motor,

language, social and cognitive development; and the emotional/behavioral domain covers the subscales: Irritability, Inflexibility and Difficulty with Routines, with 4 questions each (Fig. 1**).** Parents were also asked whether they have any concerns about their child's behavior or development.

Sampling and statistical analyses

The sample estimation was performed using the formula for analytical and experimental studies with a continuous outcome variable [18], considering the standard deviation and expected difference on the development score between groups after the intervention, obtained in a previous study [19]. The minimum of 14 participants in each group was estimated, considering the statistical power of 80% and the significance level of 5%. Twice the sample estimated was recruited to compensate for an expected loss of 50% during the intervention, according to a clinical trial with similar sample [20].

The database was built using the Epi Info ™ 3.5.1 program with double typing to enable proper consistency analysis. Statistical Package for the Social Sciences (SPSS) software version 20.0 and Stata® version 13 were used for statistical analyses.

Descriptive analyses were performed with the calculation of frequencies and measures of central tendency and dispersion. The adherence of the variables to the normal distribution was assessed using the Shapiro-Wilk test. The difference between women from omega-3 and placebo groups at the baseline, and children at birth, was evaluated using the Chi-square and Student T test.

Intention-to-treat and per-protocol analyses were conducted using the Generalized Estimating Equations (GEE) models with the Bonferroni post-hoc test, for analysis of repeated measures considering the effect of time, group and group-time interaction. The analyses were adjusted by breastfeeding practice, considering the effect of this variable on child development and behavior. A significance level of 5% was adopted in all analyses.

Results

A total of sixty pregnant women were randomized to receive placebo (n = 30) or omega-3 (n = 30). A 43.3% follow-up loss was observed (total: n = 26; omega-3: n = 14; placebo: n = 12), with no differences between the groups ($p > 0.05$). Follow-up losses are presented in Fig. 2.

Overall, capsules were well accepted by participants. The reason for dropping the study during pregnancy (n = 5) was the interruption of antenatal care at the ambulatory. All women who discontinued supplementation (n = 5) reported often forgetting to take the capsules. Only one woman reported intolerance to supplementation (placebo), presenting nausea and vomiting. Compliance was above 90% and did not differ between placebo (93.94 ± 1.14%) and omega-3 group (94.85 ± 1.22%) ($p = 0.589$). During the postpartum period, one woman asked to leave the study because of a lack of time to answer the questionnaires, and other losses (n = 10) were due to the impossibility to contact the participant.

Women who dropped the study or were excluded from analyses did not differ from those who completed the trial according to age, *per capita* income, professional occupation, education, marital status, parity, pregestational and current BMI, history of depression, pregnancy intention and fatty acids intake ($p > 0,05$) (data not shown).

Maternal characteristics of omega-3 and placebo groups at baseline are presented in Table 1. Before the intervention, there were no differences between the groups according to sociodemographic data and pregnancy

information (p > 0.05). Also, no differences were found in omega-3 intake by food consumption between groups (placebo: 1.51 ± 0.64g; omega-3: 1.69 ± 0.77g; p = 0.313) (data not shown).

Table 1
Maternal characteristics before intervention according to group.

Variables	Percent (n)		p value
	Placebo	Omega-3	
Age (years)			
20–30	66.7 (20)	70.0 (21)	0.515
31–40	33.1 (10)	30.0 (9)	
Per capita **income (minimum wage)**			
<0.5	27.6 (8)	23.1 (6)	0.782
0.5-1.0	48.3 (14)	57.7 (15)	
>1.0	24.1 (7)	19.2 (5)	
Professional occupation			
Paid work	46.7 (14)	60.0 (18)	0.702
Student	10.0 (3)	10.0 (3)	
Housewife	30.0 (9)	23.3 (7)	
Unemployed	13.3 (4)	6.7 (2)	
Marital status			
Married/Stable union	82.1 (23)	62.1 (18)	0.092
Single/Divorced	17.9 (5)	37.9 (11)	
Education			
Elementary school	6.7 (2)	10.0 (3)	0.799
High school	60.0 (18)	63.3 (19)	
Higher education	33.3 (10)	26.7 (8)	
Pregnancy intention			
Intended	51.7 (15)	41.4 (12)	0.430
Non intended	48.3 (14)	58.6 (17)	
Parity			0.196
Primiparous	60.0 (18)	43.3 (13)	
Multiparous	40.0 (12)	56.7 (17)	
Note: p-value for chi-square test. Minimum wage: ≈ $230 (2018–2019). SD: standard deviation			

Child characteristics at birth according to groups are presented in Table 2. There were no differences in sex, gestational age, route of birth and anthropometry between the groups ($p > 0.05$). Also, no differences were found in breastfeeding, exclusive breastfeeding, and breastfeeding on demand according to group during the first six months of the child's life (Table 3).

Table 2
Child characteristics at birth according to group.

Variables	Percent (n)		p value
	Placebo	Omega-3	
Sex			
Female	65.2 (15)	40.0 (8)	0.098
Male	34.8 (8)	60.0 (12)	
Gestational age			
Preterm (< 37 weeks)	4.3 (1)	5.0 (1)	0.890
Full-term (≥ 37 weeks)	95.7 (22)	95.0 (19)	
Route of birth			
Vaginal	78.3 (18)	60.0 (12)	0.193
Caesarean section	21.7 (5)	40.0 (8)	
Weight			
Adequate	82.6 (19)	100.0 (19)	0.188
Low weight	17.4 (4)	0.0 (0)	
Height			
Adequate	91.3 (21)	100.0 (19)	0.056
Low height	8.7 (3)	0.0 (0)	
Head circumference			0.265
Adequate	100.0 (19)	94.7 (18)	
Small head circumference	0.0 (0)	5.3 (1)	
Breastfed in the 1st hour of life			
Yes	82.6 (19)	75.0 (15)	0.541
No	17.4 (4)	25.0 (5)	
Skin-to-skin contact after birth			
Yes	91.3 (21)	84.2 (16)	0.480
No	8.7 (2)	15.8 (3)	
Note: p-value for chi-square test.			

Table 3
Evolution of breastfeeding practice according to group during the first six months of life.

Time	Breastfeeding Percent (n)			Exclusive breastfeeding Percent (n)			Breastfeeding on demand Percent (n)		
	Placebo	Omega-3	p value	Placebo	Omega-3	p value	Placebo	Omega-3	p value
1st month									
Yes	100.0 (18)	86.7 (13)	0.110	55.6 (10)	76.9 (10)	0.220	88.9 (16)	100.0 (13)	0.214
No	0.0 (0)	13.3 (2)		44.4 (8)	23.1 (3)		11.1 (2)	0.0 (0)	
4th month									
Yes	87.5 (14)	81.3 (13)	0.626	28.6 (4)	7.7 (1)	0.163	78.6 (11)	69.2 (9)	0.580
No	12.5 (2)	18.8 (3)		71.4 (10)	92.3 (12)		21.4 (3)	30.8 (4)	
6th month									
Yes	88.9 (16)	68.8 (11)	0.050	0.0 (0)	0.0 (0)	-	70.6 (12)	63.6 (7)	0.700
No	11.1 (2)	31.2 (5)		100.0 (17)	100.0 (11)		29.4 (5)	36.4 (4)	
Note: p-value for chi-square test.									

The comparison between groups showed no differences in the Developmental Milestones score at any time ($p > 0.05$) (Table 4). When compared to the first month, the omega-3 group showed an increase in the Developmental Milestones score at the fourth and sixth month, in both intention-to-treat and per-protocol analysis. Such increase was not observed in the placebo group.

Table 4
Intragroup and between groups comparison of child development and behavior score according to linear Generalized Estimating Equation (GEE) models.

Item	Time	Per protocol		Intention-to-treat	
		Placebo	**Omega-3**	**Placebo**	**Omega-3**
		Mean (95%CI)	**Mean (95%CI)**	**Mean (95%CI)**	**Mean (95%CI)**
Developmental milestones	1st month	14.20 (12.49–15.91)	12.78 (11.08–14.47)^{A}	14.11 (12.72–15.50)	12.76 (11.21–14.32)^{A}
	4th month	15.12 (13.85–16.40)	15.73 (14.27–17.19)^{B}	15.42 (14.26–16.59)	15.50 (14.26–16.72)^{B}
	6th month	15.20 (13.87–16.53)	16.00 (13.93–17.03)^{B}	15.36 (14.26–16.46)	15.80 (13.94–17.64)^{B}
Irritability	1st month	1.79 (0.39–3.19)	0.54 (0.03–1.05)	1.56 (0.46–2.65)	0.78 (0.11–1.44)
	4th month	1.56 (0.72–2.41)	1.43 (0.52–2.35)	1.82 (0.91–2.72)	1.35 (0.48–2.21)
	6th month	1.72 (0.56–2.87)	1.22 (0.69–1.75)	1.78 (0.86–2.70)	1.33 (0.75–1.90)
Inflexibility	1st month	2.89 (1.64–4.14)	1.56 (0.32–2.79)	2.89 (1.90–3.88)	1.60 (0.51–2.69)
	4th month	2.04 (1.15–2.93)	1.63 (0.81–2.46)	1.84 (0.99–2.69)	1.81 (0.99–2.62)
	6th month	1.66 (0.68–2.64)	1.12 (0.32–1.91)	1.59 (0.51–1.93)	1.22 (0.51–1.93)
Dificulty with routine	1st month	3.40 (2.33–4.47)^{a}	1.28 (0.10–2.46)^{b}	3.02 (2.06–3.97)^{a}	1.27 (0.24–2.30)^{b}
	4th month	3.17 (2.15–4.19)^{a}	1.45 (0.85–2.06)^{b}	2.86 (1.93–3.78)	1.72 (0.92–2.52)
	6th month	3.63 (2.15–5.11)^{a}	0.84 (0.62–2.30)^{b}	3.09 (1.85–4.33)^{a}	0.96 (0.26–2.18)^{b}

CI: confidential interval. Note: Analysis adjusted by breastfeeding practice. Different uppercase letters indicate intragroup difference and different lowercase letters indicate difference between groups, according to the post-hoc test (Bonferroni).

Regarding the Emotional/Behavioral Domain, no differences were found between groups for Irritability and Inflexibility scores at any time ($p > 0.05$). However, in per-protocol analysis, higher scores for Difficulty with Routine were observed in the placebo group when compared to omega-3 at all times, and at the first and sixth month in intention-to-treat analysis (Table 4).

Discussion

The antenatal supplementation with omega-3 did not promote differences in child development score when compared to the placebo group, however, the omega-3 group showed a significant increase in this score over time. Higher scores for Difficulty with Routine were observed in the placebo group, with no differences for Irritability and Inflexibility scores.

Our results are in line with the meta-analysis conducted by Middleton et al. [4]. According to the study, the antenatal omega-3 supplementation promoted very few differences in cognition and neurodevelopment [4].

A clinical trial conducted by Ramakrishnan et al. [7], used the Bayley Scales of Infant Development-II (BSID-II) to evaluate the effect of antenatal supplementation with algal DHA (400mg/day) on offspring development at 18 months of age. The results showed that the supplementation had no effects on child development, although DHA intake attenuated the association between home environment and psychomotor development, suggesting potential benefits for children living in home environments with reduced caregiver interactions and child stimulation [7].

Similarly, Hurtado et al [3] recruited pregnant women to take 400 mL/day of a control dairy drink or a DHA–enriched dairy drink (400mg/day) and the BSID-II was used to evaluate the infant development at 12 months. All infants presented normal neurodevelopment for the age, with no significant differences between the groups, but the antenatal supplementation showed a major effect on DHA levels in plasma and erythrocyte membranes of mothers and infants [3].

In contrast, the maternal plasma DHA concentration was associated with child development in a follow-up study conducted by Mulder et al [8]. The authors assessed 5–6 years children of mothers who received placebo or DHA (400mg/day) during pregnancy, using the second edition of Kaufman Assessment Battery for Children. Despite finding no differences between groups, maternal DHA plasma concentration was positively related to child performance on language and short-term memory [8].

The role of omega-3 fatty acids in the development of the fetal central nervous system is well described [5], however, the effect of supplementation during pregnancy remains unclear in the literature. It is noteworthy that the studies available until now differ regarding the dosage and supplement adopted, sample characteristics, intervention length and development assessment tools, therefore, to systematize the results is very challenging.

Despite the lack of differences in the development score between the groups in our trial, the increase in this score over time, observed solely in the omega-3 group, might be an indicator of differences in long-term child development that should be investigated in future studies.

Also, children of women who supplemented omega-3 during pregnancy presented less difficulty with routine in our study. Until now, no trials with omega-3 supplementation have been published using SWYC to assess infant development and behavior, which makes it difficult to compare our results with the available literature. However, it is known that alterations in child behavior are strong predictors of developmental deficits throughout childhood [17].

Additionally, the Difficulty with Routine subscale comprises mostly questions about infant sleep, and previous studies on this subject have found a similar association [21–23].

According to a meta-analysis conducted by Dai et al. [23], the omega-3 supply during pregnancy may improve infants' sleep organization and maturity, especially reducing the occurrence of active sleep [23]. Such improvement was demonstrated by Judge et al. [22], in a trial that aimed to evaluate the effect of using a functional food containing 300mg of DHA during pregnancy, on infant sleep patterning. The authors found lower arousals in quiet and active sleep in the intervention group when compared to placebo, concluding that increased antenatal intake of DHA has a beneficial impact on infant's sleep organization, and therefore in their routine [22].

The authors suggested that DHA supply during the intrauterine development may modify brain phospholipids and affect neural function, resulting in a more mature central nervous system, which is essential for a healthy sleep pattern [21–23]. Also, omega-3 intake during pregnancy is associated with an increased gestational length [4], due to the effect on prostaglandins balance involved in parturition, a fact that may impact the child neurodevelopment, and consequently improve their sleep behavior. Nevertheless, it is important to note that the studies mentioned above were conducted with newborns, and further investigations regarding the effect of antenatal omega-3 supplementation on sleep behavior of older infants are needed.

Considering the results found in our study, the use of a higher DHA dosage does not seem to increase the effect of antenatal omega-3 supplementation on child development and behavior. The use of 1440mg/day of DHA did not promote stronger effects when compared to studies that used 300-400mg/day of DHA, as presented above. The mean dietary intake of omega-3 of our sample was above the Adequate Intake (1.4g/day), proposed by Institute of Medicine [24], which reinforces the hypothesis of the supplementation being more effective for populations with omega-3 deficiency. In addition, our sample consisted of healthy pregnant women, and their offspring consequently had a low risk of inadequate neurodevelopment. However, the increase on development score over time and the lower difficult with routine, observed in the intervention group despite the breastfeeding practice, suggests that the effect of omega-3 on child development and behavior should be further investigated.

The lack of omega-3 plasma levels assessment to evaluate compliance and to explain the results of the effect is a limitation of this trial. Despite that, the relevance of the present study is highlighted, considering the investigation of the effect of a higher DHA dosage during pregnancy on child development and behavior. Also, the association of omega-3 supplementation with the sleep behavior (included in the routine) of older infants is an unprecedented result that should be further explored in the future.

In conclusion, the antenatal supplementation with a DHA dosage of 1440mg/day did not promote significant differences between groups for child development, but the omega-3 group showed an increase in this score over time. The placebo group had greater difficulty with routine than the omega-3 group, indicating a beneficial effect of antenatal supplementation on child behavior, more specifically in sleep behavior, and this association should be further explored using specific tools to assess children's sleep patterns.

Abbreviations

- CONSORT: Consolidated Standards of Reporting Trials
- DHA: docosahexaenoic acid
- EPA: eicosapentaenoic acid
- GEE: Generalized Estimating Equations
- IOM: Institute of Medicine

- ReBec: Brazilian Registry of Clinical Trials
- SWYC: Survey of Well-being of Young Children

Declarations

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Availability of data and material: Due to the nature of this research, participants of this study did not agree for their data to be shared publicly, so supporting data is not available.

Code availability: Not applicable.

Authors' contributions: Sousa TM participated in the project design, bibliographic review, data collection, statistical analysis and article writing. Santos LC acted in the project design and review of the article.

Ethics approval: This study was approved by the Research Ethics Committee under number 87705018.0.0000.5149 and registered by the Brazilian Registry of Clinical Trials (ReBec) under number U1111-1215-7952 (http://www.ensaiosclinicos.gov.br/rg / RBR-6gbzw6/).

Consent to participate: All participants signed a consent form after receiving the necessary information and expressed spontaneous interest to participate in the trial.

Consent for publication: Not applicable.

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Figures

DEVELOPMENTAL DOMAIN	
Age	**Question**
2 months *From 1 month and 0 days to 3 months, 31 days*	Makes sounds that let you know he or she is happy or upset Seems happy to see you Follows a moving toy with his or her eyes Turns head to find the person who is talking Holds head steady when being pulled up to a sitting position Brings hands together Laughs Keeps head steady when held in a sitting position Makes sounds like "ga," "ma," or "ba" Looks when you call his or her name
4 months *From 4 month and 0 days to 5 months, 31 days*	Holds head steady when being pulled up to a sitting position Brings hands together Laughs Keeps head steady when held in a sitting position Makes sounds like "ga," "ma," or "ba" Looks when you call his or her name Rolls over Passes a toy from one hand to the other Looks for you or another caregiver when upset Holds two objects and bangs them together
6 months *From 6 month and 0 days to 8 months, 31 days*	Makes sounds like "ga," "ma," or "ba" Looks when you call his or her name Rolls over Passes a toy from one hand to the other Looks for you or another caregiver when upset Holds two objects and bangs them together Holds up arms to be picked up Gets into a sitting position by him or herself Picks up food and eats it Pulls up to standing
The answers options were: (0) not yet; (1) somewhat; (2) very much. *The score could range from 0 to 20 points*	

EMOTIONAL/BEHAVIORAL DOMAIN		
Age	**Subscale**	**Question**
Children up to 18 months	**Irritability**	Does your child have a hard time being with new people? Does your child have a hard time in new places? Does your child have a hard time with change? Does your child mind being held by other people? Does your child cry a lot?
	Inflexibility	Does your child have a hard time calming down? Is your child fussy or irritable? Is it hard to comfort your child?
	Difficulty with routine	Is it hard to keep your child on a schedule or routine? Is it hard to put your child to sleep? Is it hard to get enough sleep because of your child? Does your child have trouble staying asleep?
The answers options were: (0) not at all; (1) somewhat; (2) very much. *The score for each subscale could range from 0 to 8 points.*		

Figure 1

Parts of the Survey of Well-being of Young Children (SWYC) questionnaire used in the trial.

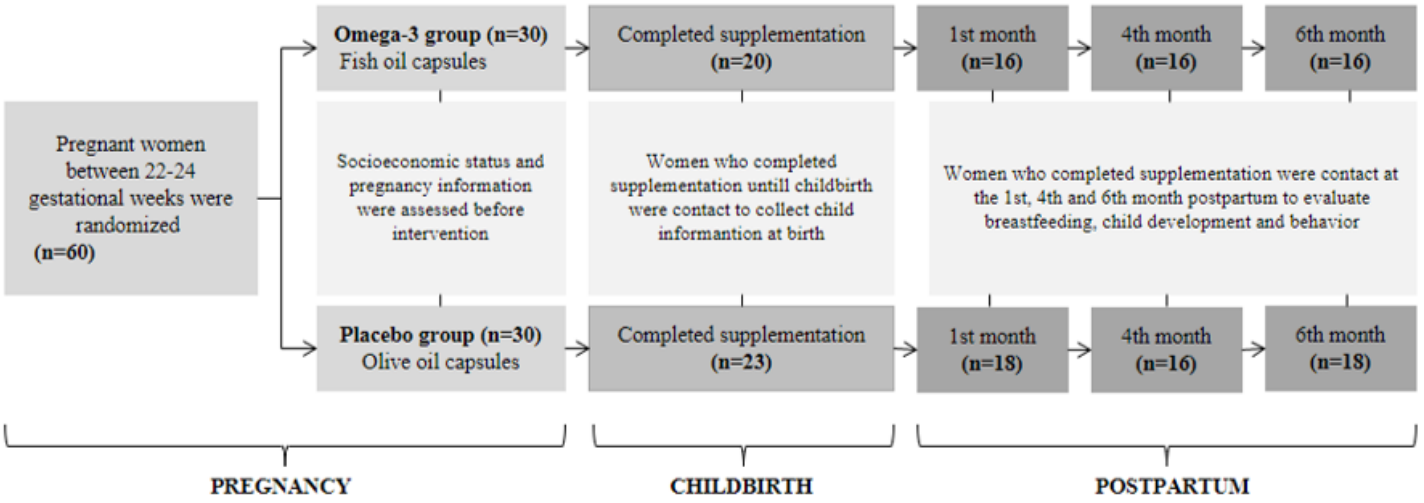

Figure 2

Diagram of trial follow-up.