



## Human Milk Lipid Profile Assessment and Influences of Mother's Diet

### STUDY SYNOPSIS

#### 1. Title

Evaluation of the lipid profile of breast milk and its variation in relation to the maternal diet.  
Randomized, controlled, blinded clinical trial.

#### 2. Eligibility (inclusion criteria)

Women within 15 post-partum days who have delivered newborns of EG $\geq$  34 weeks.  
Written informed consent given.

#### 3. Exclusion criteria

Impossibility / lack of breastfeeding will  
Contraindications to breastfeeding  
Genetic diseases / neonatal malformations  
Linguistic barriers

#### 4. Study design

Randomized, controlled, single-blinded clinical trial.

#### 5. Treatment

Nutritional indications to increase the dietary consumption of foods naturally rich in DHA and ALA.

#### 6. Scheduled number

24 women

#### 7. Primary objective

Evaluation of the lipid profile of breast milk, at time 0 (10-15 days postpartum) and at time 1 (about 3 months postpartum). Measurement of total fat (g/100 ml), saturated fatty acids, monounsaturated fatty and polyunsaturated fatty acids concentration (mg/100 ml), and specifically LA, ALA, ARA, DHA and EPA concentration (mg/100 ml) will be performed with gas chromatograph coupled to a MS / MS mass spectrometer.

Evaluation of differences at time 1 among women on a free diet and the group randomized to specific dietary advice aimed at increasing the intake of fatty acids  $\omega$ 3 (DHA, EPA and ALA) with the diet.

#### 8. Secondary objectives

Evaluation of the correlation between real dietary intake (food diary) and lipid profile of breast milk,

Evaluation of differences in milk lipid profile of mothers who gave birth to term or pre-term (34-36 weeks).

#### 9. Security parameters

anthropometric growth of the infant during the study

#### 10. Rational

As a rule, nursing mothers are not given nutritional indications aimed at improving the lipid profile of milk, despite the scientific evidence in favor of the importance of DHA in the infant's diet.

The research aims to determine the effect of a dietary counseling specifically targeted to increase the dietary intake of fatty acids  $\omega$ 3 (DHA, EPA and ALA) on the lipid profile of breast milk after 3 months; to identify effective and feasible nutrition claims for breastfeeding women.

## Statistical evaluation

### Calculation of sample size

Assuming from literature data [16] that at T0 the dosage of DHA in human milk is 0.21 – 0.1 (unit of measure) in both arms and that dietary indications lead to a 60% increase in T1 compared to T0. With a power of 80% and a significance level of 0.05, the minimum number per group is 11 women. Assuming more than 10-15% of dropouts during follow-up, the study aims to enroll 12 women per arm, for a total number of 24 subjects.

### Randomization

Randomization will be performed with the Random Number Generator of the Emilia Romagna Region Website (<http://www.servizi.regione.emilia-romagna.it/generatore/>)  
Statistical analysis plan

Results will be reported through averages and standard deviations (ds) for numerical variables with approximately normal distribution or through more robust measures such as median and inter-quartile range in case of non-normal distribution. The distributions of the categorical variables will instead be described by absolute frequencies and percentages. The two treatment groups will be compared with baseline characteristics to evaluate the balance of randomization. In this regard, the Student's t test or non-parametric equivalent will be used, Wilcoxon-Mann-Whitney test for continuous variables, while Pearson's  $\chi^2$  test or Fisher's exact test for categorical variables may be used.

For the evaluation of the primary endpoint, tests for repeated measurements will be used: t-test or Wilcoxon test for paired data depending on the distribution of the variables. Furthermore, the relationship between DHA variation in diet and the consequent variation of fatty acids contained in breast milk will be investigated, through correlation measures such as Pearson's index or Spearman's non-parametric correlation. Finally, the variation of DHA in human milk will be compared in the subgroups defined by the other variables collected as the pre-term birth, in order to identify those factors that can act as effect modifiers of the diet. The same statistical tests described above will be used for these comparisons. Statistical significance is set at a level  $\alpha = 0.05$  and analyzes will be performed using Stata 13 statistical software (StataCorp, 2013, College Station, TX).