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Randomized controlled trial of 4.0 mg versus 0.4 mg folic acid supplementation: Follow-up of children at 1 year of age

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ABSTRACT

Objective and study design: This 1-year follow-up study reports the results on the health status, visits to the paediatrician and hospitalizations of children born from the women recruited in the main randomized controlled trial (RCT) that investigated the effect of periconception folic acid (FA) supplementation of 4.0 mg/day on reducing adverse reproductive outcomes.
Methods: The health status of livebirths was evaluated by a trained health care provider (HCP) through a phone interview with the paediatrician (at 1–3–12 months of age) and with the parents (at 12 months of age), using a structured data collection form.
Results: Information at 1 year of life could be obtained for 347/376 (92.3 %) newborns included in the original RCT. No statistically significant differences were observed between the two groups regarding weight, health problems, hospitalizations from birth to 1 year of life and developmental milestones, as well as accesses to the emergency ward and parents'worries. Breastfeeding differed significantly at 1, 3 and 12 months of life, with higher proportion of exclusive breastfeeding in the 4.0 mg FA Group.
Conclusion: The findings suggest that the periconception FA supplementation of 4.0 mg/day versus 0.4 mg/day, does not affect the health status and hospitalizations from birth to 1 year of life, as well as normal child's developmental milestones at 1 year of life. The increase in exclusive breastfeeding in the 4.0 mg FA group needs further investigation.

Abbreviations: AIFA, Italian Medicines Agency; BMI, body mass index; FA, folic acid; HCP, health care provider; INMA, Infancia y Medio Ambiente; NTD, neural tube defect; RCT, randomized controlled trial; SD, standard deviation; SGA, small for gestational age.

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1. Introduction

Folic acid (FA) supplementation before conception and in early pregnancy is recommended to women planning a pregnancy to reduce the risk of neural tube defects (NTDs) [1,2]. In 2015 a Cochrane review [3] confirmed that FA prevents the first and second time occurrence of NTDs, but did not find a clear effect on other birth defects (namely cleft palate, cleft lip, congenital cardiovascular defects) as previously suggested [4,5]. Moreover, the available literature offers an uncertain impact of FA supplementation on prevention of other adverse reproductive outcomes [6–18]. Observational studies indicated an association between greater level of folate during pregnancy and higher birthweight [8] and fewer cases of small for gestational age (SGA) [9,10] and preterm delivery [11], although such findings were not reported in other investigations [12,13]. To date, the relationship between folate insufficiency and the onset of placenta mediated diseases, such as spontaneous abortion, preterm birth, fetal growth restriction, preeclampsia [15,16], abruptio placentae [17] and stillbirth [4,18] is not fully understood. Furthermore, the problem of the most appropriate dose of FA is still open [19,20].

In a previous multicenter double-blind randomized controlled trial (RCT) [21], we investigated the effects of a higher dose of periconception FA on reducing adverse reproductive outcomes. One thousand sixty women (aged 18–44 years and planning a pregnancy within 12 months) were randomly assigned to receive 4.0 mg or 0.4 mg of FA daily, from randomization before pregnancy until the 12th gestational week. The doses of FA were based on evidence of efficacy, recommendations regarding occurrence and recurrence of NTDs [1–3], and studies on other adverse pregnancy outcomes [22,23]. The RCT did not show any advantage of FA 4.0 mg daily supplementation on the occurrence of congenital malformations, but it was associated with fewer spontaneous abortions, SGA and preterm births, and with a better composite outcome including one or more adverse pregnancy outcomes [21].

The primary prevention of these adverse pregnancy outcomes in the general population is a crucial policy priority. Considering that the most appropriate dose and timing of FA are still under debate, postnatal health status and somatic development of children should also be considered when assessing the effects of FA supplementation and the differences between the groups, in obstetric and perinatal RCTs.

This study reports the results of 1-year follow-up in terms of health status, visits to the paediatrician and hospitalizations, in children born from the women recruited in the main RCT.

2. Material and methods

This is the follow-up study of an original RCT comparing 4.0 mg versus 0.4 mg FA daily supplementation. The study design has already been published elsewhere [21]. Briefly, children included in this study were born to women recruited in the Italian Folic Acid Trial Study, an RCT to evaluate the effect of periconception FA supplementation of 4.0 mg/day compared to 0.4 mg/day standard dose on the occurrence of congenital malformations and other adverse reproductive outcomes. The follow-up study was included in the original protocol which was approved by the Ethic Committee (n. 1591) [21]. Informed consent was obtained from all subjects involved. The study (clinical trial number FARM6KWTCT, 16/07/2008) was conducted according to the guidelines of the Declaration of Helsinki.

The health status of livebirths was evaluated by a trained health care provider (HCP) through a phone interview with the paediatrician using a structured data collection form (at 1–3–12 months of age) and a phone interview with the parents (at 12 months of age). During the interviews with the paediatrician, the HCP collected information about the child's health status, visits to the paediatrician and hospitalizations, any diagnosis of malformations, breastfeeding (at 1–3–12 months of age) and inquired the paediatrician about the regularity of the child's developmental stages (at 12 months of age). During the interview with the parents, the HCP investigated the health status of the baby, any access to the emergency ward, and any worries about the health and growth of the baby.

The follow-up started in September 2010 and was completed in June 2017. Two hundred and four newborns were in the 4.0 mg FA Group, included three couples of twins. One hundred and seventy-two newborns were in the 0.4 mg FA Group, included six couples of twins. Twenty-nine babies (7.7 %) were lost to follow-up, 14 in the 4.0 mg FA Group and 15 in the 0.4 mg FA Group, respectively (Fig. 1).

The centiles of weight at 1 and 12 months were defined according to WHO child-growth-standards [24].

Statistical analysis was performed using SAS 9.4 (SAS Institute, Inc, Cary, NC, USA). Categorical variables were summarized as frequency and percentage, and compared between two groups using Chi-square test or Fisher's exact test. Numerical variables were summarized as mean (standard deviation, SD) or median (range), and compared between two groups using Student t test or Mann-Whitney test. In order to take into account the effect of potential confounding factors multivariate analysis was also conducted for the end point "normal child's developmental milestones" (terms included in the analysis are specified in the foot note of the Tables 1–3).

All tests were 2-sided and a p-value less than 0.05 was considered statistically significant.

All researchers involved in the analyses were masked to the allocation group.

3. Results

Overall, information at 1 year of life could be obtained for 347/376 (92.3 %) newborns included in the original RCT (190/204, 93.1 % in 4.0 mg FA Group and 157/172, 91.3 % in 0.4 mg FA Group).

Maternal characteristics and mode of delivery were not statistically different between the two groups (Table 1), while FA 4.0 mg/day maternal supplementation was associated with higher body length (mean 50.3 versus 49.7 cm, $p = 0.03$) and head circumference (mean 34.5 versus 33.9 cm, $p < 0.001$) of the newborns (Table 1).

During the follow-up, breastfeeding showed a statistically significant difference at 1, 3 and 12 months of life ($p = 0.05$, 0.01 and 0.02 respectively, Table 2), with higher proportion of exclusive breastfeeding in the 4.0 mg FA Group.

Weight at 1 month and weight at 12 months did not significantly differ between the two groups ($p = 0.06$ and $p = 0.63$ respectively). Babies < 10th percentile were different between the two groups at 1 month (9 in the 4.0 mg FA Group versus 18 in the 0.4 mg FA Group, $p = 0.02$), but not at 12 months ($p = 0.80$) (Table 3).

No statistically significant differences were observed between the two groups regarding health problems and hospitalizations from birth to 1 year of life (Table 3).

Normal developmental milestones were not observed in 4/183 (2.2 %) children in the 4.0 mg FA Group (psychomotor developmental delay, motor developmental delay, height and weight growth retardation, weight loss) and in 9/150 (6.0 %) children in the 0.4 mg FA Group (psychomotor developmental delay, motor developmental delay, weight growth retardation, hyperactivity and multisystemic developmental disorder). This difference was not statistically significant even after taking into account the potential confounding effect of breastfeeding, weight and head circumference in the multivariate analysis ($p = 0.219$) (Table 3).

Of note, children in the 4.0 mg FA Group had less visits to the paediatrician in their first 12 months of life, with a median of 7 visits in the 4.0 mg FA Group versus 9 in the 0.4 mg FA Group, ($p = 0.02$).

When interviewed at 12 months, parents of the two groups reported similar accesses to the emergency ward ($p = 0.99$) and worries about the health and growth of the baby ($p = 0.53$) (Table 4).

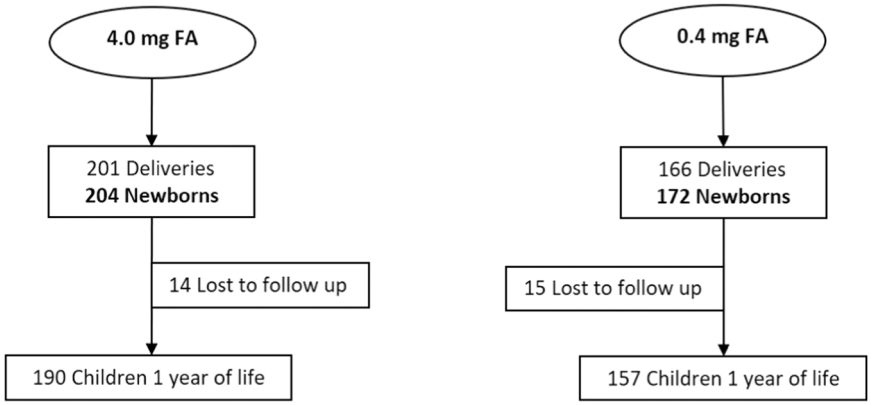

Fig. 1. Flow-chart of follow-up participants according to study groups.

Table 1
Maternal, delivery and neonatal characteristics according to study groups.

Maternal and delivery characteristics	4.0 mg FA Group ($n = 201$)	0.4 mg FA Group ($n = 166$)	p-value[c]
Sociodemographic characteristics			
Maternal age, years: mean (SD)	31.9 (4.3)	32.0 (3.7)	0.74
Education, years: mean (SD)	15.1 (3.5)	14.6 (3.5)	0.24
Lifestyle/Personal habits			
Cigarette smokers, no. (%)	29 (14.4)	24 (14.5)	0.99
Alcohol drinkers, no. (%)	99 (49.3)	86 (51.8)	0.63
BMI[a], no. (%)			
< 18.50	17 (8.5)	10 (6.0)	
18.50–24.99	158 (78.6)	130 (78.3)	0.55
≥ 25.00	26 (12.9)	26 (15.7)	
Reproductive history			
Previous pregnancies, no. (%)			
0	107 (53.2)	91 (54.8)	0.76
≥ 1	94 (46.8)	75 (45.2)	
Delivery			
Mode of delivery, no. (%)			
Spontaneous vaginal delivery	99 (50.3)	77 (47.2)	
Induced vaginal delivery	37 (18.8)	30 (18.4)	
Operative vaginal delivery	12 (6.1)	12 (7.4)	0.21
Elective caesarean section	26 (13.2)	13 (8.0)	
Emergency caesarean section	23 (11.7)	31 (19.0)	
Missing	4	3	
Neonatal characteristics	**4.0 mg FA Group ($n = 204$)**	**0.4 mg FA Group ($n = 172$)**	**p-value[d]**
Body length[b], cm,			
Mean (SD)	50.3 (2.3)	49.7 (2.7)	0.03
Missing	17	16	
Head circumference[b], cm,			
Mean (SD)	34.5 (1.5)	33.9 (1.4)	< 0.001
Missing	37	39	

FA: folic acid, SD: standard deviation.
[a] The body mass index (BMI) is the weight in kilograms divided by the square of the height in meters.
[b] Term and preterm, single and twin delivery.
[c] Chi-square.
[d] *t*-test.

Table 2
Breastfeeding according to study groups.

Characteristics	4.0 mg FA Group ($n = 201$)	0.4 mg FA Group ($n = 166$)	p-value[a]
First month, no. (%)[b]			
Exclusive	142/181 (78.5)	97/146 (66.4)	
Mixed	26/181 (14.4)	31/146 (21.2)	0.05
Formula	13/181 (7.2)	18/146 (12.3)	
Third month, no. (%)[c]			
Exclusive	132/182 (72.5)	94/147 (64.0)	
Mixed	30/182 (16.5)	19/147 (12.9)	0.01
Formula	20/182 (11.0)	34/147 (23.1)	
Twelve months, no. (%)[d]			
Exclusive	96/181 (53.0)	55/145 (37.9)	
Mixed	21/181 (11.6)	15/145 (10.4)	0.02
Formula	64/181 (35.4)	75/145 (51.7)	

FA: folic acid.
[a] Chi-square.
[b] Data not available in 40 subjects.
[c] Data not available in 38 subjects.
[d] Data not available in 41 subjects.

4. Discussion

The 1-year follow-up study of 92.3 % of children born from women recruited in the Italian Folic Acid Trial Study, showed that babies < 10th percentile were different between the two groups at 1 month but not at 12 months of life. Maternal supplementation of 4.0 mg/day FA was also associated with higher head circumference and body length of the newborns. No significant differences emerged between the two groups in relation to health problems and hospitalizations from birth to 1 year of life, as well as normal child's developmental milestones at 1 year of life. To our knowledge, no data are currently available on children at 1 year of age from follow-up RCTs on maternal periconception supplementation of 4.0 mg/day FA.

The effect of periconception multivitamin supplementation (including 0.8 mg of FA) on postnatal development was studied in a RCT comparing the use of a multivitamin tablet with placebo-like trace elements [25]. Of 4122 liveborn infants, 3356 were examined after the eighth month of life (mean age 11 months). Somatic development (body weight, body length, and head circumference) did not show a significant difference between the two groups. Mental and behavioural development was also similar in the two groups. There was no significant difference in the rates of serious or chronic disorders between the study groups except for atopic dermatitis, which was reported more often in the group receiving multivitamins (15 versus 4 cases). Our study did not confirm this difference between groups.

The literature offers other related studies which unfortunately are not useful for a comparison due to limitations or heterogeneous study features. In a previous UK study [26], 96 children aged 2–5 years (mean 3.6 years) and 91 children aged 7–10 years (mean 8.8 years) were assessed clinically for somatic and mental behavioural development after periconception supplementation with a multivitamin (including 0.36 mg of FA) and minerals. This study group had no appropriate control. No harmful effect was detected and a possible beneficial effect was noted on growth.

Thereafter, several follow-up studies conducted in this area evaluated health status and development of children whose mothers had received FA supplementation but different study design, timing and dose

Table 3
Health status of children at 1 and 12 months of age according to study groups.

Characteristics of children[o]	4.0 mg FA Group ($n = 204$)	0.4 mg FA Group ($n = 172$)	p-value[g]
Weight at 1 month, g: mean (SD)[a]	4134.5 (694.3)	3985.3 (757.8)	0.06
Weight at 12 months, g: mean (SD)[b]	9768.7 (1263.9)	9701.8 (1299.4)	0.63
Weight < 10th percentile at 1 month, no. (%)[a]	9/182 (4.9)	18/151 (11.9)	0.02
Weight < 10th percentile at 12 months, no. (%)[b]	3/184 (1.6)	2/154 (1.3)	0.80
Visits to the paediatrician from birth to 12 months of age: median (range)[c]	7 (6–10)	9 (7–11)	0.02
Health problems from birth to 12 months of age, no. (%)[d]:			
Respiratory	24/190 (12.6)	29/157 (18.5)	0.18
Hearing	8/190 (4.2)	2/157 (1.3)	0.12
Sight	2/190 (1.1)	2/157 (1.3)	0.99
Language	0/190 (0.0)	2/157 (1.3)	0.20
Atopic dermatitis	5/190 (2.6)	4/157 (2.5)	0.99
Hospitalization from birth to 12 months of age, no. (%)[e]	32/180 (17.7)	31/148 (20.9)	0.47
Normal child's developmental milestones, no. (%)[a,f]	179/183 (97.8)	141/150 (94.0)	0.07*

[a] Data not available in 43 subjects.
[b] Data not available in 38 subjects.
[c] Data not available in 41 subjects.
[d] Respiratory health problems included respiratory tract infections and bronchospasm; hearing problems included ear infections; sight problems included vision problems and conjunctivitis; language problems included slight language delay.
[e] Data not available in 48 subjects.
[f] Normal developmental milestones were not observed in 4/183 (2.2 %) children in 4.0 mg FA Group (psychomotor developmental delay, motor developmental delay, height and weight growth retardation, weight loss) and in 9/150 (6.0 %) children in 0.4 mg FA Group (psychomotor developmental delay, motor developmental delay, weight growth retardation, hyperactivity and multisystemic developmental disorder).
[g] Chi-square (frequencies) or *t*-test in case of mean (SD).
[o] Term and preterm, single and twin delivery.
* We have also computed a multivariate analysis including terms for breastfeeding (formula/mixed versus exclusive), weight (underweight versus normal weight) and head circumference (in continuum): the p-value was 0.219 for the 4.0 mg FA compared with the 0.4 mg FA treatment group.

Table 4
Parents interview at 12 months of age according to study groups.

	4.0 mg FA Group ($n = 204$)	0.4 mg FA Group ($n = 172$)	p-value[b]
Access to the emergency ward, no. (%)[a]			
No	122/188 (64.9)	102/157 (65.0)	0.99
Yes	66/188 (35.1)	55/157 (35.0)	
Worries about health and growth of baby, no. (%)[a]			
No	181/188 (96.3)	149/157 (94.9)	0.53
Yes	7/188 (3.7)	8/157 (5.1)	

FA: folic acid.
[a] Data not available in 31 subjects.
[b] Chi-square.

of FA intake, timing and goals of the studies make the comparison among studies difficult [27–33].

Considering that real-life data suggest that many pregnant women use high doses of FA, up to 5 mg/day and even more in several European countries [34,35], the results of this 1-year follow-up study of children are of particular interest.

Regarding the effects of high doses of FA supplementation in early pregnancy on child neurodevelopment at 18 months of age [36], the prospective mother-child cohort 'Rhea' Study in Greece investigated whether high doses of FA supplementation in early pregnancy were associated with child neurodevelopment at 18 months of age. Compared with non-users, daily intake of 5 mg supplemental FA was associated with a 5-unit increase on the scale of receptive communication and a 3.5-unit increase on the scale of expressive communication.

The multicenter prospective mother-child cohort INMA Project [37] recruited pregnant women from 4 areas of Spain. Children whose mothers used FA supplement dosages higher than 5000 μg/day during pregnancy had a statistically significantly lower mean psychomotor scale score after the first year of life, than children whose mothers used a recommended dosage of FA supplements (400–1000 μg/day).

At birth, we found that 4.0 mg FA supplementation was associated with increased head circumference when compared to 0.4 mg FA. The difference corresponded to around 2 weeks of growth [38]; furthermore, 4.0 mg FA supplementation was associated with increased body length and, in the original RCT [21], with higher birthweight ($p = 0.01$), compared to 0.4 mg FA. According to our results, the Generation R Study [39] suggested an independent, modest association between maternal folate concentrations in early pregnancy and fetal head growth, but more research is needed to identify whether specific brain regions are affected and whether effects of folate on fetal head growth influence children's long-term functioning. Furthermore, previous results of the Generation R Study [40] showed a significant association for preconception initiation of maternal FA use with fetal head and abdominal circumference in the second and third trimester.

Interestingly, we found that 4.0 mg FA supplementation was associated with increased exclusive breastfeeding at 1–3–12 months of age when compared to 0.4 mg FA. We do not have any reasonable explanation for this finding, which may be evaluated in further studies.

Moreover, children in the 4.0 mg FA Group had less visits to the paediatrician in their first 12 months of life. This difference, although statistically significant, does not appear clinically relevant, and can be reasonably explained by a higher number of infants < 10th percentile and less exclusive breastfeeding in the 0.4 mg FA Group in comparison with the 4.0 mg FA Group.

Regarding the FA intake, the adherence was assessed in the original RCT by interviewing the participants and by counting the tablets that were returned by women at each visit. Adherence in pregnant women was good [21], without any differences between the two groups. Moreover, maternal determinants of periconception FA intake were considered in the RCT and found balanced between groups [21,41].

Recently paternal characteristics such as age, education, occupational class and country of origin were associated with maternal periconception FA supplementation [42]. In the original RCT, paternal age and education were balanced between groups (data not shown).

Our follow-up study has some limitations that should be considered. First, follow-up data are limited to 1 year of age, but this time frame was designed in the original RCT. Second, specific parameters regarding development and neurocognitive development were not available in the interview with the paediatrician. However, we acknowledge that data at this early age may be poorly predictive of subsequent development. Third, data on head circumference and body length after the first month of age would have been interesting, but the original RCT was not designed to collect such data. However, the head circumference in the two groups was in the range of the Italian head circumference growth charts [43].

Furthermore, the RCT was unable to sustain the desired rate of recruitment and to achieve the estimated sample size. Despite a specific enrolment strategy, we believe that the lack of preconception counselling in Italy and routine referral setting for women planning a natural pregnancy were likely to be the causes of such a low rate in patient recruitment. When planning future studies, we recommend the establishment of services specifically dedicated to women/couples of

childbearing age who plan to have a baby in the short to medium term.

Relevant strengths of our study include the high adherence to follow-up at 1 year after birth. With regard to methodological issues, the follow-up of infants is an important component of the assessment in obstetric and perinatal RCTs; to our knowledge, no data are currently available on children at 1 year of age from follow-up RCTs on maternal periconception supplementation of 4.0 mg/day FA. Finally, the study design of the original RCT on the doses and timing of FA use, as well as the participation of paediatricians in the follow-up study and their regular involvement in the assessment of the child's health status, add strength and interest to the findings.

5. Conclusions

Our findings suggest that periconception FA supplementation, before pregnancy until the 12th gestational week, of 4.0 mg/day versus 0.4 mg/day, does not affect the health status and hospitalizations from birth to 1 year of life, as well as normal child's developmental milestones at 1 year of life. The increase in exclusive breastfeeding in the 4.0 mg FA Group needs further investigation.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

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Author contributions

R.B., P.M., F.P. and D.T. designed the main study and the follow-up. F.F. and the General Coordination Center collected the data. F.P., S.C. and F.C. planned and conducted the statistical analyses. R.B. and F.C. drafted the manuscript. D.T., F.M., P.M. and F.P. revised the manuscript. All authors read and approved the final version of the manuscript for publication.

CRediT authorship contribution statement

Cipriani Sonia: Formal analysis, Data curation.

Conflict of interest statement

The authors declare no conflict of interest.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved during the EC meeting n. 1591 (clinical trial number FARM6KWTCT, 16/07/2008).

Declaration of Competing Interest

We know of no conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

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Membership of the Italian Folic Acid Trial Study Group

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