



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

Renata Bortolus^{a,1}, Francesca Filippini^{a,2}, Sonia Cipriani^{b,3,*}, Daniele Trevisanuto^{c,4}, Federico Marchetti^{d,5}, Pierpaolo Mastroiacovo^{e,6}, Fabio Parazzini^{b,f,7}, Francesco Cavallin^{g,8}, on behalf of the Italian Folic Acid Trial Study Group⁹

^a Office for Research Promotion, Verona University Hospital, Verona 37134, Italy

^b Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Ca' Granda Ospedale Maggiore Policlinico, Milan 20122, Italy

^c Department of Woman's and Child's Health, University of Padua, Padua 35128, Italy

^d Department of Pediatrics, Santa Maria Delle Croci Hospital, Ravenna, Italy

^e International Centre on Birth Defects-ICBD, Rome 00195, Italy

^f Department of Clinical Sciences and Community Health, University of Milan, Milan 20122, Italy

^g Independent Statistician, Solagna 36020, Italy

ARTICLE INFO

Keywords:
Children
Development
Folic acid
Follow-up
Health problems
Breastfeeding

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.

* Correspondence to: Gynecology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, via della Commenda 12, Milan 20122, Italy.

E-mail address: sonia.cipriani@policlinico.mi.it (S. Cipriani).

¹ <https://orcid.org/0000-0003-3857-4133>.

² <https://orcid.org/0000-0001-5884-8635>.

³ <https://orcid.org/0000-0002-0530-499X>.

⁴ <https://orcid.org/0000-0002-7126-5298>.

⁵ <https://orcid.org/0000-0001-6202-169X>.

⁶ <https://orcid.org/0000-0002-8318-8664>.

⁷ <https://orcid.org/0000-0001-5624-4854>.

⁸ <https://orcid.org/0000-0002-0831-3971>.

⁹ Membership of the Italian Folic Acid Trial Study Group is provided in the Acknowledgement section.

<https://doi.org/10.1016/j.eurox.2025.100370>

Received 22 November 2023; Received in revised form 9 January 2025; Accepted 3 February 2025

Available online 4 February 2025

2590-1613/© 2025 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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).

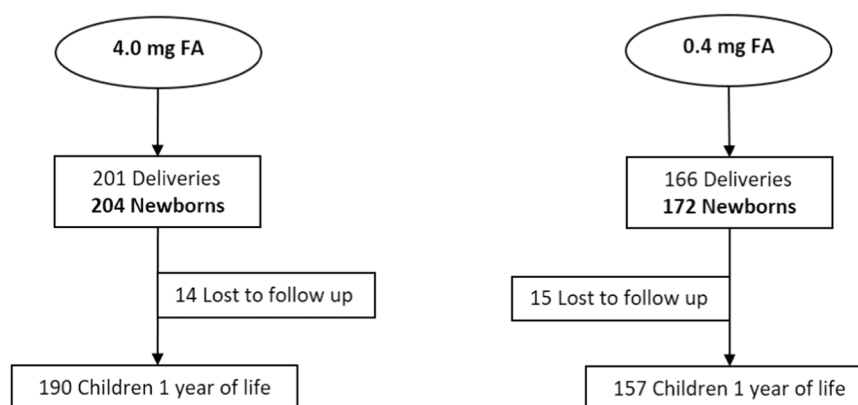


Fig. 1. Flow-chart of follow-up participants according to study groups.

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
<u>Sociodemographic characteristics</u>			
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
<u>Lifestyle/Personal habits</u>			
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)	0.55
18.50–24.99	158 (78.6)	130 (78.3)	
≥ 25.00	26 (12.9)	26 (15.7)	
<u>Reproductive history</u>			
Previous pregnancies, no. (%)			
0	107 (53.2)	91 (54.8)	0.76
≥ 1	94 (46.8)	75 (45.2)	
<u>Delivery</u>			
Mode of delivery, no. (%)			
Spontaneous vaginal delivery	99 (50.3)	77 (47.2)	0.21
Induced vaginal delivery	37 (18.8)	30 (18.4)	
Operative vaginal delivery	12 (6.1)	12 (7.4)	
Elective caesarean section	26 (13.2)	13 (8.0)	
Emergency caesarean section	23 (11.7)	31 (19.0)	
Missing	4	3	
<u>Neonatal characteristics</u>			
	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.

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

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)	0.05
Mixed	26/181 (14.4)	31/146 (21.2)	
Formula	13/181 (7.2)	18/146 (12.3)	
Third month, no. (%) ^c			
Exclusive	132/182 (72.5)	94/147 (64.0)	0.01
Mixed	30/182 (16.5)	19/147 (12.9)	
Formula	20/182 (11.0)	34/147 (23.1)	
Twelve months, no. (%) ^d			
Exclusive	96/181 (53.0)	55/145 (37.9)	0.02
Mixed	21/181 (11.6)	15/145 (10.4)	
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.

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 ^a	4.0 mg FA Group (n = 204)	0.4 mg FA Group (n = 172)	P-value ^e
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.

Funding information

This work was supported by a grant from the Italian Medicines Agency (AIFA) within the independent drug research program 2006 (clinical trial no. FARM6KWTCT, 16/07/2008). The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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.

Acknowledgements

We thank the women, couples, parents, babies, Association Spina Bifida Italy, Veneto, clinical staff who participated in this trial, and the members of the Safety Monitoring Board for their work. The trial is dedicated to Margherita, a girl with spina bifida, died in 2018, at the age of 8 years.

Membership of the Italian Folic Acid Trial Study Group

General Coordination Center: Renata Bortolus, Francesca Filippini, Erika Rigotti, Francesca Rech Morassutti, Antonella Noya di Lannoy (Verona University Hospital, Verona), Pierpaolo Mastriacovo, Emanuele Leoncini (Alessandra Lisi International Centre on Birth Defects and Prematurity-ICBD, Rome), Daniele Trevisanuto (Department of Woman's and Child's Health, University of Padua), Fabio Parazzini (Department of Woman, Newborn and Child, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico-IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan and Department of Clinical Sciences and Community Health, Università degli studi di Milano, Milan). Experimental Medicine Management: Alessandra Compagni, Silvia Manfrè, Federica Todino, Marilisa Coati (Verona University Hospital, Verona). Randomization Centers and Study Investigators: Verona University Hospital-Borgo Roma, Verona - Giovanni Zanonato, Anna Angeli, Elena Cavaliere, Elena Mantovani; Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan - Claudia Scarduelli, Edgardo Somigliana, Federica Alagna; Gallarate Hospital - Elena Cesari; S. Bonifacio Hospital - Debora Balestreri, Elisa Caloi; Verona University Hospital-Borgo Trento, Verona - Maria Teresa Zenorini, Greta Cherubini; Vicenza Hospital - Giuliano Zanni, Chiara Vernier; Family Planning Service, Villorba - Nedelia Minisci; Family Planning Service, Padua - Anna Casaro; General Practitioner, Schio - Gian Pietro Piazza; Azienda Ospedaliera of Padua - Erich Cosmi, Silvia Visentin; Family Planning Service, Verona - Patrizia Rosi; Family Planning Service, Martellago - Maria Grazia Salvato; General Practitioner, Malo - Chiara Tresso; Thiene/Santorso Hospital - Gianfranco Jorizzo, Gaetana Cirelli; Legnago Hospital - Achille Morandini, Paola Zanini; Family Planning Service, Verona - Graziella Mortaro; Conegliano Hospital - Andrea Baffoni, Michaela Bertezolo; Bassano del Grappa Hospital - Paola Lanza, Anna De Toni; Family Planning Service, Bassano del Grappa - Simonetta Marinangeli; General Practitioner, Verona - Laura Valotto; Luigi Sacco Hospital, Milan - Irene Cetin, Arianna Laoreti; Family Planning Service, Montebelluna - Carlo Failli; ARNAS Garibaldi Nesima Hospital, Catania - Giuseppe Ettore, Sebastiano Bianca; Bussolengo Hospital - Giorgia Negrini; General Practitioner, Sovizzo - Renato Ruffini; Family Planning Service, Vigonovo - Anna Sandri; Valdagno Hospital - Claudia Guaraldi; Family Planning Service, Mira - Marina Matterazzo; General Practitioner, Verona - Angelo Guido; General Practitioner, Dueville - Enrico Ioverno; Family Planning Service, San Pietro di Stra - Alessandra Schiavo; Adria Hospital - Enrico Di Mambro; Family Planning Service, Oderzo - Maurizia Marzolini.

References

- [1] MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the MRC vitamin study. *Lancet* 1991;338:131–7.
- [2] Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992;327:1832–5.
- [3] De-Régil LM, Peña-Rosas JP, Fernández-Gaxiola AC, Rayco-Solon P. Effects and safety of periconceptional oral folate supplementation for preventing birth defects. *Cochrane Database Syst Rev* 2015;CD007950.
- [4] Czeizel AE, Dobo M, Vargha P. Hungarian cohort-controlled trial of periconceptional multivitamin supplementation shows a reduction in certain congenital abnormalities. *Birth Defects Res A Clin Mol Teratol* 2004;70:853–61.
- [5] Botto LD, Olney RS, Erickson JD. Vitamin supplements and the risk for congenital anomalies other than neural tube defects. *Am J Med Genet* 2004;125C:12–21.
- [6] Lassi ZS, Salam RA, Haider BA, Bhutta ZA. Folic acid supplementation during pregnancy for maternal health and pregnancy outcomes. *Cochrane Database Syst Rev* 2013;CD006896.

- [7] Balogun OO, Lopes KDS, Ota E, Takemoto Y, Rumbold A, Takegata M, et al. Vitamin supplementation for preventing miscarriage. *Cochrane Database Syst Rev* 2016.
- [8] Fekete K, Berti C, Trovato M, Lohner S, Dullemeijer C, Souverein OW, et al. Effect of folate intake on health outcomes in pregnancy: a systematic review and meta-analysis on birth weight, placental weight and length of gestation. *Nutr J* 2012;11: 75.
- [9] Li N, Li Z, Ye R, Liu J, Ren A. Impact of periconceptional folic acid supplementation on low birthweight and small-for-gestational-age infants in China: a large prospective cohort study. *J Pediatr* 2017;187:105–10.
- [10] Zhang Q, Wang Y, Xin X, Zhang Y, Liu D, Peng Z, et al. Effect of folic acid supplementation on preterm delivery and small for gestational age births: a systematic review and meta-analysis. *Reprod Toxicol* 2017;67:35–41.
- [11] Li B, Zhang X, Peng X, Zhang S, Wang X, Zhu C. Folic acid and risk of preterm birth: a meta-analysis. *Front Neurosci* 2019;13:1284.
- [12] Martinussen MP, Bracken MB, Triche EW, Jacobsen GW, Risnes KR. Folic acid supplementation in early pregnancy and the risk of preeclampsia, small for gestational age offspring and preterm delivery. *Eur J Obstet Gynecol Reprod Biol* 2015;195:94–9.
- [13] Saccone G, Berghella V. Folic acid supplementation in pregnancy to prevent preterm birth: a systematic review and metaanalysis of randomized controlled trials. *Eur J Obstet Gynecol Reprod Biol*. Vol. 199; 201. p. 76–81.
- [14] Gaskins AJ, Chavarro JE. Diet and fertility: a review. *Am J Obstet Gynecol* 2018; 218:379–89.
- [15] Wen SW, White RR, Rybak N, Gaudet LM, Robson S, Hague W, et al. Effect of high dose folic acid supplementation in pregnancy on pre-eclampsia (FACT): double blind, phase III, randomised controlled, international, multicentre trial. *BMJ* 2018; 362:k3478.
- [16] Bulloch RE, Lovell AL, Jordan VMB, McCowan LME, Thompson JMD, Wall CR. Maternal folic acid supplementation for the prevention of preeclampsia: a systematic review and meta-analysis. *Paediatr Perinat Epidemiol* 2018;32: 346–57.
- [17] Nilsen RM, Vollset SE, Rasmussen SA, Ueland PM, Daltveit AK. Folic acid and multivitamin supplement use and risk of placental abruption: a population-based registry study. *Am J Epidemiol* 2008;167:867–74.
- [18] Gaskins AJ, Rich-Edwards JW, Hauser R, Williams PL, Gillman MW, Ginsburg ES, et al. Maternal prepregnancy folate intake and risk of spontaneous abortion and stillbirth. *Obstet Gynecol* 2014;124:23–31.
- [19] Wald NJ, Morris JK, Blakemore C. Public health failure in the prevention of neural tube defects: time to abandon the tolerable upper intake level of folate. *Public Health Rev* 2018;39:2.
- [20] Berry RJ. Lack of historical evidence to support folic acid exacerbation of the neuropathy caused by vitamin B12 deficiency. *Am J Clin Nutr* 2019;110:554–61.
- [21] Bortolus R, Filippini F, Cipriani S, et al. Efficacy of 4.0 mg versus 0.4 mg folic acid supplementation on the reproductive outcomes: a randomized controlled trial. *Nutrients* 2021;13:4422.
- [22] Czeizel AE, Bánhidy F. Folic acid supplementation and risk reduction in preterm birth. *Am J Clin Nutr* 2011;94:1651–2.
- [23] Vila-Nova C, Wehby GL, Queirós FC, Chakraborty H, Félix TM, Goco N, et al. Periconceptional use of folic acid and risk of miscarriage—findings of the Oral Cleft Prevention Program in Brazil. *J Perinat Med* 2013;41:461–6.
- [24] (<https://www.who.int/tools/child-growth-standards/standards/weight-for-age>) [Accessed 22 February 2023]
- [25] Czeizel AE, Dobó M. Postnatal somatic and mental development after periconceptional multivitamin supplementation. *Arch Dis Child* 1994;70:229–33.
- [26] Holmes-Siedle M, Dennis J, Lindenbaum RH, Galliard A. Long term effects of periconceptional multivitamin supplements for prevention of neural tube defects: a seven to 10 year follow up. *Arch Dis Child* 1992;67:1436–41.
- [27] Wehby GL, Murray JC. The effects of prenatal use of folic acid and other dietary supplements on early child development. *Matern Child Health J* 2008;12:180–7.
- [28] Julvez J, Fortuny J, Mendez M, Torrent M, Ribas-Fito N, Sunyer J. Maternal use of folic acid supplements during pregnancy and four year-old neurodevelopment in a population-based birth cohort. *Paediatr Perinat Epidemiol* 2009;23:199–206.
- [29] Roza SJ, van Batenburg-Eddes T, Steegers EA, et al. Maternal folic acid supplement use in early pregnancy and child behavioural problems: the Generation R Study. *Br J Nutr* 2010;103:445–52.
- [30] Julvez J, Forns M, Ribas-Fito N, Torrent M, Sunyer J. Attention behavior and hyperactivity and concurrent neurocognitive and social competence functioning in 4-year-olds from two population-based birth cohorts. *Eur Psychiatry* 2011;26: 381–9.
- [31] Roth C, Magnus P, Schjølberg S, et al. Folic acid supplements in pregnancy and severe language delay in children. *JAMA* 2011;306:1566–73.
- [32] Steenweg-de Graaff J, Roza SJ, Steegers EA, et al. Maternal folate status in early pregnancy and child emotional and behavioral problems: the Generation R Study. *Am J Clin Nutr* 2012;95:1413–21.
- [33] Compañ-Gabucio LM, Torres-Collado L, García-de la Hera M, et al. Association between the use of folic acid supplements during pregnancy and children's cognitive function at 7–9 years of age in the INMA cohort study. *Int J Environ Res Public Health* 2022;19:12123.
- [34] Pastor-Valero M, Navarrete-Muñoz EM, Rebagliato M, et al. Periconceptional folic acid supplementation and anthropometric measures at birth in a cohort of pregnant women in Valencia, Spain. *Br J Nutr* 2011;105:1352–60.
- [35] Papadopoulou E, Stratakis N, Roumeliotaki T, et al. The effect of high doses of folic acid and iron supplementation in early-to-mid pregnancy on prematurity and fetal growth retardation: the mother–child cohort study in Crete, Greece (Rhea study). *Eur J Nutr* 2012;52:327–36.
- [36] Chatzi L, Papadopoulou E, Koutra K, et al. Effect of high doses of folic acid supplementation in early pregnancy on child neurodevelopment at 18 months of age: the mother–child cohort ‘Rhea’ study in Crete, Greece. *Public Health Nutr* 2012;15:1728–36.
- [37] Valera-Gran D, García de la Hera M, Navarrete-Muñoz EM, et al. Infancia y Medio Ambiente (INMA) Project. Folic acid supplements during pregnancy and child psychomotor development after the first year of life. *JAMA Pediatr* 2014;168: e142611.
- [38] Bergerat M, Heude B, Taine M, et al. Head circumference from birth to five years in France: new national reference charts and comparison to WHO standards. *Lancet Reg Health Eur* 2021;5:100114.
- [39] Steenweg-de Graaff J, Roza SJ, Walstra AN, et al. Associations of maternal folic acid supplementation and folate concentrations during pregnancy with foetal and child head growth: the Generation R Study. *Eur J Nutr* 2017;56:65–75.
- [40] Timmermans S, Jaddoe VW, Hofman A, Steegers-Theunissen RP, Steegers EA. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. *Br J Nutr* 2009;102:777–85.
- [41] Nilsen RM, Leoncini E, Gastaldi P, Allegri V, Agostino R, Faravelli F, et al. Prevalence and determinants of preconception folic acid use: an Italian multicenter survey. *Ital J Pediatr* 2016;42:65.
- [42] Mortensen JHS, Øyen N, Nilsen RM, Fomina T, Tretli S, Bjørge T. Paternal characteristics associated with maternal periconceptional use of folic acid supplementation. *BMC Pregnancy Childbirth* 2018;18:188.
- [43] Bertino E, Spada E, Occhi L, Coscia A, Giuliani F, Gagliardi L, et al. Neonatal anthropometric charts: the Italian neonatal study compared with other European studies. *J Pediatr Gastroenterol Nutr* 2010;51:353.