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The Higher Rate of Multiple Births After Periconceptional Multivitamin Supplementation: An Analysis of Causes

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Abstract. A randomized controlled trial of periconceptional multivitamin supplementation (including 0.8 mg folic acid – see the Materials and Methods section for the precise composition of the multivitamin and trace-element supplementation) was carried out for at least 28 days before conception. The trial was continued until at least until the second missed menstrual period to test the effectiveness of this new primary preventive method in the reduction of neural tube defects. However, other pregnancy outcomes were also evaluated. Of a total of 5,502 pregnant women, 4,846 births were analysed in the final data base. The rate of multiple births was significantly higher in the multivitamin group (3.8%) than in the placebo-like trace-element control group (2.7%), and in both groups exceeded the multiple birth rate of 2.2% in the Hungarian population at large. 7.3% of women in the multivitamin and 7.9% of women in the trace-element groups had received ovarian stimulation treatment (mainly clomiphene) for hormonal dysfunctions, eg. anovulation. Nonetheless, our study showed that periconceptional multivitamin supplementation, with or without ovarian stimulation, increases the rate of multiple births.

Key words: Multiple births, Periconceptional multivitamin supplementation, Ovarian stimulation

INTRODUCTION

In a recent publication the United States Public Health Service recommended: "All women of childbearing age in the United States who are capable of becoming pregnant should consume 0.4 mg of folic acid per day for the purpose of reducing their risk of having a pregnancy affected with spina bifida or other neural tube defects" [2]. Other countries, eg., the UK and the Netherlands have also made similar recommendations. This advice was based on the findings of the Hungarian randomized controlled trial of

periconceptional multivitamin supplementation, including 0.8 mg of folic acid [5], and some observational studies [1, 17, 19, 24]. Intervention studies also indicated that periconceptional supplementation with multivitamins including folic acid [21, 22] or folic acid alone [11, 18] can reduce the re-occurrence of neural tube defects. The expected wider use of this primary preventive method makes the study of other effects of periconceptional multivitamin/folic acid supplementation imperative. In the Hungarian randomized controlled trial [7], the rate of multiple births was higher after periconceptional multivitamin supplementation, a finding which is evaluated here in detail.

MATERIALS AND METHODS

The Hungarian randomized controlled trial of periconceptional multivitamin/trace element supplementation [3], which formed part of the Hungarian Optimal Family Planning Programe, was launched on February 1, 1984 and ended on April 30, 1992. Participants' pregnancy outcomes were evaluated until the end of April, 1993. The criteria of eligibility for participation in the trial, the method of randomization and monitoring of compliance with supplement intakes, and the criteria of full, partial and no supplementation (women who conceived before starting or during the first month of supplement use) have been presented previously [5]. Study participants' compliance in taking the supplementation was ascertained by interview, by checking of basal body temperature records and by counting unused tablets when the tablet boxes were returned. Women who took the supplementation course for 28 days before conception and at least until the date of the second missed menstrual period were defined as having received a full supplementation course. Women who received a partial course were defined as those who failed to take the supplementation for more than one day during the periconceptional period (although in general, these women only failed to take the supplementation for a few days). Women who conceived before or during the first month of administration were considered not to have received supplementation. In this study, our intention-to-treat analysis regarded the group of women we originally intended to treat, while our treatment analysis concerned those participants remaining after the exclusion of the unsupplemented groups.

The Hungarian Optimal Family Programme involves three main steps, each performed and supervised by qualified nurses: a reproductive health check, a three-month period of preparation for conception, and the safe-guarding of early pregnancy. Of the three items contained in the three-month pre-conceptional preparatory period, two were important for the purposes of our study. Firstly, the measurement of basal body temperature, and advice and assistance in the detection of hormonal dysfunctions, eg., anovulation and luteal insufficiency. Women with hormonal dysfunctions were examined further and/or treated. Secondly, the participants were advised to take a single multivitamin tablet ie., ELEVIT Pronatal^{R} (Roche) or trace element tablet daily, for one month before planned conception, and until the 12th week of gestation, based on the date of their menstrual period. The composition of the multivitamin tablet was as follows: 6000 IU of vitamin A until the end of 1989 and 4000 IU thereafter, 1.6 mg of B_1, 1.8 mg of B_2, 19 mg of nicotinamide, 2.6 mg of B_6m, 10 mg of calcium pantothenate, 0.2 mg of biotin, 4 μg of B_{12}, 100 mg of calcium, 125 mg of phosphorus, 100 mg of magnesium,

60 mg of iron, 1 mg of copper, 1 mg of manganese, and 7.5 mg of zinc. Each trace-element tablet contained 1 mg of copper, 1 mg of manganese, 7.5 mg of zinc and 7.5 mg of vitamin C.

Women participating in the trial completed certificates recording their pregnancy outcomes, including infants' dates of birth, sex, weight, gestational age, whether singletons, twins, triplets or higher-order multiples, and in particular whether infants presented any birth defects. These certificates were signed by the women's doctors and sent to our staff. All deliveries, terminations and recorded fetal deaths occurred in Hungarian obstetrical in-patient clinics. Considerable effort was made to assure that the pregnancy outcomes of all women with confirmed pregnancies were evaluated. If a completed certificate had not been received by one month after the expected date of delivery, a letter was mailed to the trial participant, asking her to return the completed certificate and/or discharge summary. If there was no response, one of our coworkers visited her at home. Only if these efforts failed to elicit of response was the woman deemed to have dropped out from the study. All multiple births families were interviewed in 1992 and 1993, and the type of twins was evaluated on the basis of sex, placentation, anthropometric features and, if necessary, of genetic markers.

Statistical evaluation of our data was perfomed using the two-tailed chi-square test, and relative risk (RR) with 95% confidence intervals (CI) were calculated.

RESULTS

Of 5,502 gravidae, pregnancy outcomes could be evaluated in 5,453 cases (Table 1). Trial participants' failure to return their completed certificates was attributed to changes of address or stays abroad. Demographic factors were similar in the two study groups.

Of the 4767 pregnancies ending in live- and stillbirths, 78 multiple gestations were

Table 1 - Pregnancies in the groups studied according to supplementation course and demographic characteristics

Supplement	Confirmed pregnancies No.	Dropouts No.	Dropouts %	Evaluated pregnancies No.	Maternal age $\bar{X}$	Maternal age S.D	Proportion of primiparae (%)
Multivitamin							
Full course	1980	11	0.6	1969	27.0	3.3	88.0
Partial course*	573	5	0.9	568	26.9	3.6	86.4
No supplement	266	10	3.8	256	26.1	3.2	95.3
Total	2819	26	0.9	2793	26.9	3.4	88.3
Trace element							
Full course	1914	5	0.3	1909	27.0	3.3	89.4
Partial course	552	5	0.9	547	26.6	3.4	89.4
No supplement	217	13	6.0	204	26.2	3.5	96.1
Total	2683	23	0.9	2660	26.9	3.4	89.9

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recorded (It was generally not possible to recognize multiple pregnancies in the case of miscarriages, ectopic pregnancies and pregnancies confirmed by a positive pregnancy test and/or ultrasound scan, with no clinical evidence of pregnancy). Pregnancy was terminated in 16 women after the diagnosis of fetal defects in the second trimester. All these fetuses were singletons. The data from the group of women we initially intended to treat showed that the difference in the multiple pregnancy rate ($\chi_1^2 = 2.13$; $p = 0.15$; RR = 1.4, 95% CI = 0.87–2.26) was not significant between the multivitamin and trace-element groups. However, the rate of multiple births (Table 2) in the multivitamin group significantly exceeded the rate of the trace-element group ($\chi_1^2 = 4.48$; $p = 0.03$; RR = 1.42, 95% CI = 1.01–1.98). All but one multiple pregnancy resulted in twins: the exception was one set of boy-boy-girl triplets. After exclusion of the unsupplemented groups, a more obvious difference was apparent between the study groups in both the rate of multiple pregnancies ($\chi_1^2 = 2.94$; $p = 0.09$) and of multiple births ($\chi_1^2 = 6.14$; $p = 0.01$; RR = 1.53, 95% CI = 1.08–2.16).

Sex and type of twin-pairs in the group of women we intended to treat are shown in Table 3. The proportion of like-sex twins in the multivitamin group somewhat exceeds that of the trace-element group. However, the difference between the two groups is not significant ($\chi_1^2 = 1.29$; $p = 0.26$). The proportion of mono- and dizygotic twin-pairs was also not significantly different between the multivitamin and trace-element groups ($\chi_1^2 = 0.42$; $p = 0.52$). A positive family history of twins only occurred in one trace-element supplemented pregnancy. The demographic characteristics of mothers who delivered twins did not differ significantly between the groups studied. The mean birthweight and gestational age of twins reflected the well-known characteristics of twin

Table 2 - Number and rate of twin pregnancies and twin (live- and still-) births in the groups studied

Intention-to-treat analysis	Multivitamin group	Trace-element group
Total informative[+] pregnancies	2421	2346
Twin pregnancies	46[x]	32
%	1.90	1.36
Total births	2468	2378
Twin births	93[x]	64
%	3.77	2.69
Excluding unsupplemented group		
Total informative[+] pregnancies	2198	2170
Twin pregnancies	44[x]	29
%	2.00	1.34
Total births	2243	2199
Twin births	89	58
%	3.97	2.64

Key: × = including one boy-boy-girl triplet
+ = informative pregnancies included all pregnancies ending in live- or stillbirths

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Table 3 - Sex and type of twin-pairs, and demographic characteristics of women bearing twins

Variable	Multivitamin No.	Multivitamin %	Trace element No.	Trace element %
Sex				
Boy-boy	18	40.0	10	31.3
Girl-girl	13	28.9	8	25.0
Total like-sex	31	68.9	18	56.3
Boy-girl	14	31.1	14	43.7
Total	45	100.0	32	100.0
Type of zygosity				
Monozygotic[x]	14	31.8	8	25.0
Dizygotic	30	68.2	24	75.0
Mean(±S.D.) maternal age (yr)	26.4±2.8		26.4±3.1	
Primiparous %	90.0		93.8	
Mean (±S.D.) birth weight (g)				
Twin A	2507±467		2513±421	
Twin B	2426±548		2447±402	
Mean (±S.D.) gestation age (week)	37.6±2.0		37.3±1.8	

Key: x = one stillborn boy-liveborn boy twin-pair had no zygosity diagnosis

births, and also did not differ significantly between the multivitamin and trace- element groups.

The overall perinatal mortality rate was 2.5% (4/157). All stillbirths and neonatal deaths occurred in the group receiving the full multivitamin course (Table 4). However, the difference was not significant between the study groups ($\chi^2_{Yates} = 1.36$; Fisher $p_2 = 0.15$). Of 13 cases with congenital abnormalities, two concordant twin-pairs with congenital dislocation of the hip and one concordant twin-pair with congenital postural deformity were found. There was no difference in the rate of congenital abnormalities between the groups studied ($\chi^2_1 = 0.17$; $p = 0.68$). The occurrence of minor anomalies (eg., epicanthal fold, hemangioma, and umbilical hernia) was examined too, but no obvious difference in their rate ($\chi^2_1 = 1.67$; $p = 0.20$) and pattern was observed between the multivitamin and trace-element groups.

The use of infertility drugs was also evaluated. Unsupplemented women were excluded from this analysis because they had not undergone a 3-month pre-conceptional preparatory period, including the measurement of basal body temperature to detect hormonal dysfunction. This was generally followed with clomiphene and/or other infertility drug treatments. (One dizygotic male-female twin pair conceived after their mother received clomiphene treatment was recorded in the unsupplemented trace-element group, however. Of 2,198 and 2,170 'informative' pregnancies ending in live- or stillbirths, 161 (7.3%) and 171 (7.9%) of mothers were treated with infertility drugs in the multivitamin and trace-element groups respectively ($\chi^2_1 = 0.48$; $p = 0.49$). Clomiphene citrate

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Table 4 - Perinatal mortality, congenital abnormalities and minor anomalies in the multiples born in each group studied

Group	No. of multiple births	Late fetal death No.	Neonatal mortality No.	Congenital abnormalities No.	%	Minor anomalies No.	%
Multivitamin							
Full course	69	3[x]	1[xx]	6[□]	8.7	9	13.0
Partial course	20	0	0	1[□□]	5.0	10	50.0
No supplement	4	0	0	0	0.0	0	0.0
Total	93	3	1	7	7.5	19	20.4
Trace element							
Full course	48	0	0	4°	8.3	3	4.3
Partial course	10	0	0	1°°	10.0	3	30.0
No supplement	6	0	0	1°°°	16.7	2	33.3
Total	64	0	0	6	9.4	8	12.5

Key: [x] Male twin A with birthweight of 1000g
Male twin B of dizygotic unlike-sex twin pair with birthweight of 1950g
Female twin B of dizygotic like-sex twin-pair with birthweight of 1000g
[xx] Male twin B with birthweight of 1600 g died at 10 days of age
[□] Congenital dislocation of hip - 4; patent ductus arteriosus - 1; undescended testis -1
[□□] Congenital inguinal hernia - 1
[°] Congenital dislocation of hip - 2; congenital postural deformity - 2
[°°] Congenital dislocation of hip - 1
[°°°] Congenital pyloric stenosis - 1

(Clostilbegyt[R]) with or without other infertility drugs was used to treat the great majority of women: 141 (6.4%) in the multivitamin and 143 (6.6%) in the trace-element groups ($\chi_1^2 = 0.05$; $p = 0.82$) (Table 5). In the remaining cases, patients were treated with bromocriptine (Bromocriptin[R]) (16 vs 19), choriogonadotropin (Choriogonin[R]) (3 vs 5) or menopausal gonadotropin (Pergonal[R]) (1 vs 4).

Table 5 - Multiple pregnancies (N) and total pregnancies (n) in the fully and partially supplemented study groups with or without clomiphene treatment

Supplementation		Ovarian stimulation +	Ovarian stimulation −	Total
Multivitamin	N/n	19/141	25[x]/2057	44[x]/2198
	%	13.5	1.2	2.0
Trace element	N/n	12/143	17/2027	29/2170
	%	8.4	0.8	1.3
Total	N/n	31/284	42/4084	73/4368
	%	10.9	1.0	1.7

Key: [x] including one triplet

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The rate of multiple pregnancies was nearly 11 times higher in women who had received clomiphene treatment ($\chi_1^2 = 284.1$; $p < 0.001$). The multiple pregnancy rate was higher in women treated with a combination of clomiphene and multivitamins than in those treated with clomiphene and trace elements; however, the difference between the two groups was not significant ($\chi_1^2 = 1.89$; $p = 0.17$; RR = 1.70, 95% CI = 0.75–3.90). The increased rate of multiple pregnancies in the group of women who received multivitamins, but who had not been treated with clomiphene, while representing a noteworthy trend, was also not statistically significant ($\chi^2 = 1.42$; $p = 0.23$; RR = 1.45, 95% CI = 0.75–2.82). In addition, the increase in the multiple pregnancy rate is similar in the group of women receiving the supplement alone and in that receiving the supplement with clomiphene. However, if the multiple birth rate is calculated, the differences between the subgroup containing those women not treated with clomiphene ($\chi_1^2 = 3.15$; $p = 0.076$; RR = 1.48, 95% CI = 0.94–2.35) and that containing those treated with the drug ($\chi_1^2 = 3.40$; $p = 0.065$; OR = 1.70, 95% CI = 0.97–2.43) were nearer to the statistically significant level.

As expected, after ovarian stimulation the proportion of boy-girl and dizygotic twin-pairs increased (Table 6). However, this trend achieved the statistically significant level in the mono- and dizygotic twin ratio (0 vs 12) of the trace-element group ($\chi^2_{Yates} = 7.80$; $p = 0.5$), but not in the multivitamin group ($\chi_1^2 = 0.04$; $p = 0.84$).

Table 6 - Sex and type of twin-pairs in the fully and partially supplemented study groups with or without ovarian stimulation (OS)

Variable	Multivitamin				Trace element			
	No OS		OS		No OS		OS	
	No.	%	No.	%	No.	%	No.	%
Sex								
Boy-boy	11	44.0	6	31.6	5	29.4	4	33.3
Girl-girl	7	28.0	5	26.3	6	35.3	1	8.3
Total Like-sex	18	72.0	11	57.9	11	64.7	5	41.7
Boy-girl	7[x]	28.0	8	42.1	6	35.3	7	58.3
Total	25	100.0	19	100.0	17	100.0	12	100.0
Type of zygosity								
Monozygotic	7[xx]	29.2	5	26.3	8	47.1	0	0.0
Dizygotic	17	70.8	14	73.7	9	52.9	12	100.0

Key: [x] including one boy-boy-girl triplet
[xx] one twin-pair had no zygosity diagnosis

DISCUSSION

In the 1980s, the multiple birth rate in Hungary was 2.2% Both multivitamin and trace-element groups had a higher multiple birth rate in the Hungarian randomized controlled

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trial. However, the rate of multiple births (twins in all but one case) was significantly higher in the multivitamin than the trace-element group. There a 1.1–1.3% absolute and 42–50% relative increase in multiple births resulted from the intention-to-treat and 'treatment' (after the exclusion of unsupplemented groups) analyses. This finding may be of clinical importance, because multiple births are often associated with higher perinatal risk, which can be reduced by an early diagnosis and appropriate care and/or treatment [10, 20].

Four different groups of factors connected with the higher rate of multiple births in the Hungarian randomized controlled trial of perinatal mutivitamin supplementation were considered: (i) differences in demographic and genetic characteristics between women in the multivitamin and trace-element groups; (ii) the possible effect of periconceptional multivitamin supplementation on the prenatal selection rate; (iii) the effect of ovarian stimulation on the conception rate; and (iv) the effect of multivitamin including folic acid supplementation on the multiple birth rate.

With respect to (i) above, no demographic (maternal age and parity) or genetic differences (positive family history of DZ twins) were found between the two study groups, and (ii) was excluded, because the fetal death rate was somewhat but not significantly higher in the multivitamin group [5]. The higher multiple conception and birth rates in the Hungarian randomized controlled trial of perinatal multivitamin supplementation seems to be most plausibly explained by the effect of ovarian stimulation (iii). Indeed, the results of the trial confirmed the well-known multiple-pregnancy-inducing effect of clomiphene.

In the Hungarian Optimal Family Planning Programme, the measurement of basal body temperature during the 3-month preparation-for-conception period detected obvious hormonal dysfunction (anovulation and luteal insufficiency) in 10.4% of cases [6]. The majority of these were treated with clomiphene, which explains the relatively high proportion of women in our trial who received this treatment (6.5%), compared to the Hungarian gravid population at large. However, the significantly higher rate of multiple births after multivitamin supplementation cannot be explained by more extensive use of ovarian stimulation in this subgroup, because its rate was similar in the trace-element group.

Regarding (iv) above, a somewhat but not significantly higher rate of multiple pregnancy was also observed in the multivitamin supplemented group not receiving clomiphene treatment. This is interesting, because a higher rate of multiple births has been reported after periconceptional folate treatment in animals. One group of authors observed a drastic decrease in serum folates between weaning and mating and also during mating in multiparous sows [13, 14]. Such a drop in serum folates, which could be prevented by i.m. injections of folic acid, resulted in larger litter sizes. This finding has been confirmed elsewhere: folic acid supplementation of sows' diets (through corn soybean meal) increased the total (11.2 vs 10.2) and live pigs born (10.8 vs 9.9) per litter, ie., also the conception rate [12]. The same authors subsequently showed that high levels of serum folates also stimulated ovulation: the average litter size was 12 piglets per litter, compared to 10.5 for sows with low levels of serum folates [15]. This finding was also later confirmed by a team of authors using a different method whereby folic acid supplementation was complemented by ovarian stimulation: a single injection of 1,250 IU of pregnant mare serum gonadotropin the day after weaning, increased the ovulation rate by 41% and, as one would expect, litter size [23].

Thus, about half of the increase in the multiple birth rate of participants in the Hungarian randomized controlled trial of periconceptional multivitamin supplementation could be explained by the frequent use of ovarian stimulation due to anovulation and/or luteal insufficiency. (This method needs some revision however, following the discovery of granulosa-cell tumours in 12 patients after ovarian stimulation treatment with clomiphene citrate and/or gonadotrophins [25]). The remaining multiple birth rate increase in the multivitamin group may be the effect of the multivitamin including folic acid treatment. The synergetic effect of multivitamin supplementation and clomiphene treatment, and the effect of the multivitamin supplementation alone, resulted in an increase in multiple births in this group which was 61% higher than that in women in the trace-element groups receiving ovarian stimulation. However, for those women who did not receive clomiphene treatment, a 50% higher rate of multiple births was observed in the multivitamin than in the trace-element group ($\chi_3^2 = 150.06$; $p < 0.001$). The difference is significant (see Table 5). Another biological effect of periconceptional multivitamin supplementation was that it reduced the well-known dizygotic twinning effect of ovarian stimulation, particularly of clomiphene.

Neural tube defects in the offspring of mothers who received clomiphene treatment have been reported [8, 9]. Of the 284 women treated with clomiphene in the Hungarian randomized trial, no offspring had neural tube defects. Similar results were also found by a previous case-control study [16]. The association between twinning and neural tube defects has been studied from several aspects [9]. In general, there appears to be no association between the prevalence at birth of neural tube defects and twinning in different geographical regions. The twinning rate has been observed to be higher and the prevalence at birth of neural tube defects lower in the black than in the white population in the USA, however [9].

In conclusion, in the Hungarian Optimal Family Programme, the occurrence of multiple births was higher after periconceptional multivitamin supplementation. The effect needs further research, but may be explained mainly by two factors: the synergetic effect of the combination of multivitamins and clomiphene, and the effect of periconceptional multivitamin supplementation alone.

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