Efficacy of Multiple Micronutrient Supplementations on Child Health: Study Design and Baseline Characteristics

Natalya Bilenko MD MPH[1,2,4], Ilana Belmaker MD MPH[3,4], Hillel Vardi[2,4] and Drora Fraser PhD[1,2]

[1]Department of Epidemiology and Health Services Evaluation, [2]Abraham International Center for Health and Nutrition, and [3]Community Health Division, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel
[4]Ministry of Health Regional Office, Southern Region, Beer Sheva, Israel

ABSTRACT: **Background:** The rates of anemia in children in southern Israel are high despite the current prevention strategy. A daily dose of "Sprinkles" (SuppleForte™, Heinz, Canada), a micronutrient home supplementation, was proven effective for the treatment of anemia worldwide.

Objectives: To assess the efficacy of Sprinkles, a novel supplementation formulation, in the primary prevention of anemia in infants who have free access to health care services.

Methods: A two-arm open-labeled cluster randomized controlled clinical trial was performed in 6 month old Bedouin and Jewish infants. The Sprinkles arm received sachets with iron, vitamins A and C, folic acid and zinc, and the control arm received standard treatment (liquid iron and vitamins A and D). The infants were from families attending Mother and Child Health clinics during 2005–2007. Intervention and follow-up were conducted for babies aged 6–12 months. Health outcomes (hematologic and nutritional indicators, growth parameters, morbidity rates) were evaluated at 12 and 18 months.

Results: The final study population numbered 621 infants (328 Bedouin and 293 Jewish); of the parents approached 88.5% agreed to participate. Hemoglobin › 11 g/dl was found in 55% of Bedouin and 40% of Jewish infants ($P < 0.01$). Bedouin infants had significantly lower serum concentration of iron, folic acid and zinc. All background, hematologic and micronutrient indicators were similar in the two study arms except for a slightly but not clinically significant difference in hemoglobin and hematocrit levels in Bedouins.

Conclusions: Our findings indicate the need to improve the micronutrient status of infants living in the Negev. A cluster randomized trial in MCH clinics is a feasible option.

IMAJ 2010; 12: 342–347

KEY WORDS: "Sprinkles" (SuppleForte™, Heinz, Canada), anemia, micronutrient deficiencies, infants, Bedouin

Nutrient deficiencies of iron, vitamin A, folic acid and zinc are prevalent worldwide, especially in children from low income areas [1]. Almost two-thirds of the deaths of young children around the world are related to nutritional deficiencies [2]. Both protein-energy malnutrition and micronutrient deficiencies increase the risk of morbidity and mortality from infectious diseases [3]. In addition, the effects of nutritional deficiencies in childhood may include impaired rates of physical and cognitive development [4] as well as increased risk of poor health in adulthood [5,6]. It was recently suggested that "Sprinkles" (SuppleForte™, Heinz, Canada), a novel supplementation containing iron and other micronutrients, may be the most appropriate strategy to improve the nutritional status of infants with micronutrient deficiencies [7]. The Sprinkles formulation comprises single-serve sachets for home food fortification with iron that is encapsulated with a soya-based hydrogenated lipid, vitamins A, C and D, folic acid and zinc. Supplementation with Sprinkles does not conflict with breastfeeding and is compatible with the introduction of age-appropriate foods because it can be mixed into complementary weaning foods including homemade foods [8]. Sprinkles have been tested worldwide and were found to be an effective, easy to use and highly acceptable method for secondary prevention of iron-deficiency anemia [9]. The main goal of our study was to assess the efficacy of Sprinkles supplementation for primary prevention of iron and other micronutrient deficiencies in well-nourished pediatric populations with a high rate of anemia [10,11] and low socioeconomic level [12].

SUBJECTS AND METHODS

This study was a two-arm cluster randomized open-labeled controlled clinical trial. Enrollment in the study was carried out between July 2005 and September 2007 in 15 local Mother and Child Health clinics. Infants were included only after their parents gave written informed consent. The trial population was recruited from families of infants attending Ministry of Heath MCH clinics for routine well-baby examination and vaccinations. The primary practitioner in MCH clinics is a public health nurse with medical backup rendered by an obstetrician and a pediatrician. The public health nurse is responsible for providing guidance to pregnant women and children as well

MCH = Mother and Child Health

as health education regarding recommended health behavior; she also performs screening tests and refers pregnant woman and children to other health care facilities for laboratory tests that cannot be performed at the MCH clinic. She is responsible for follow-up and outreach in a population that does not always comply with the recommendations, and for referring any woman or child with abnormal signs to specialists for evaluation and treatment.

The allocation to treatment groups was performed by MCH clinics (randomization unit). Within each population group (Bedouins and Jews) we randomly and sequentially selected MCH clinics in order to reach the necessary sample size, based on the annual number of newborns attending the clinic. Fifteen clinics, of the 40 available, were selected using a random numbers table. Infants aged 5–7 months receiving food supplementation with or without breastfeeding were included in the study. Excluded were infants with Hb < 10 g/dl; chronic hematologic, immunologic, metabolic or malabsorption disorders; major congenital malformations; or infants participating in another study. In addition, Bedouin families residing outside permanent settlements were excluded from the study because of the intensive contact needed for follow-up procedures.

INTERVENTIONS

The study arm received sachets of Sprinkles for age 6 to 12 months. Each sachet contained 12.5 mg iron (ferrous fumarate), 5 mg zinc, 300 µg vitamin A, 7.5 µg vitamin D, 150 µg folic acid, and 50 mg ascorbic acid. These values reflect the 1999–2001 Dietary Reference Intakes stipulated by the U.S. Institutes of Medicine. None of the components exceeded the amounts recommended by Israel's Ministry of Health. Infants in the control arm received 6 drops of iron as iron (III) hydroxide polymaltose complex per day (15 mg active iron). In addition, they received 300 µg of vitamin A and 10 µg vitamin D (water-based formula) as recommended by the Ministry of Health.

FOLLOW-UP

The study arms received their assigned monthly treatment at enrollment and subsequently at monthly visits from age 6 months to age 12 months [Appendix 1]. At these encounters, a research assistant conducted a personal interview on breastfeeding practices, eating patterns, health status, morbidity episodes, health services utilization (primary care, emergency room, hospitalizations), use of supplements, satisfaction and reported possible side effects of the treatments. In addition, the research assistant counted the remaining sachets and examined the level of liquids in the iron and vitamin A and D bottles. They replenished treatment supplies and encouraged parents to continue using the supplementations. At age 12 months during an MCH clinic routine visit, a personal interview was conducted and blood was drawn. At the 18 month visit (another routine MCH clinic visit), an interview on breastfeeding practices, infant diet, morbidity episodes and use of health care services was conducted, and venous blood was obtained. At ages 6, 12 and 18 months during MCH clinic visits, nutritional evaluation using a Short Food Frequency Questionnaire for iron intake and energy assessment was performed. This FFQ was based on a list of food items that had been offered or consumed the day before and reported by 160 mothers of infants aged 6–24 months. The FFQ was designed according to the methods described previously [14]. All interviews were performed by trained interviewers speaking Hebrew or Arabic, as appropriate. Venous blood was obtained by a pediatrician. For a complete blood count, 2 ml of blood were drawn into the tubes with EDTA. For plasma zinc, folic acid and iron determinations, 3 ml of blood were drawn (without EDTA). All samples were transported on ice (0–4°C) to the laboratory within 2 hours. Hemoglobin and other indicators of complete blood count were analyzed using the ADVIA® 120 Hematology system. Zinc level was assessed using atomic absorption spectrometry. Plasma iron was measured colorimetrically after acidification and precipitation of plasma proteins. Serum ferritin was measured using enzyme-linked immunosorbent assay. The methods remained constant throughout the study. Laboratory evaluation of vitamins A, C and D was not available for technical reasons.

The written informed consent of the parents to enroll their children in this study included the parents' agreement to access the infants' medical records both from the MCH clinic and Soroka Medical Center. The study design was approved by the Institutional Review Board of Soroka. The study was registered in the Clinical Trials registry of the U.S. National Institutes of Health (Study NCT00276198)

DEFINITIONS

Mild anemia (Hb < 11 g/dl), moderate anemia (Hb < 10 g/dl), low hematocrit (Hct < 32.9%), low mean corpuscular volume (MCV < 77 fl), low mean corpuscular hemoglobin (< 26 pg), low mean corpuscular hemoglobin concentration (< 32 g/dl), high red blood cell distribution width (RDW >14%), low serum ferritin (SF < 15 µg/L), low transferrin saturation (TS < 16%), low zinc (< 60 µg/dl), low folic acid (< 2.7 ng/ml) (Recommendations to prevent and control iron deficiency in the United States, Centers for Disease Control and Prevention, MMWR, 1998). Under-nutrition (< -2 weight-for-height z-scores), stunting (< -2 height-for age z-score) using the CDC reference population [15].

OBJECTIVES

The overall objective of this study was to compare the efficacy of Sprinkles (Sprinkles arm) with the standard treatment supplied to the families (control arm). Differences between the arms were examined for the following parameters: iron

FFQ = Food Frequency Questionnaire

deficiency indicators (Hb, Hct, MCV, RDW, SF, TS), other micronutrient deficiencies (zinc, folic acid), health indicators (morbidity, health care services utilization), and compliance with the supplementation. Our primary aim was to determine the difference in hemoglobin level between infants in the Sprinkles and control arms at 12 and 18 months. In addition, we wished to compare the two study arms for: a) mean levels of Hct, MCV, RDW, iron, SF and TS; b) mean levels of folic acid and zinc level; c) mean of weight-for-height and height-for age z-scores; d) incidence rates of reported infectious disease morbidity; e) reported primary health care clinic and emergency room visits, and reported and recorded hospitalizations; f) compliance with recommended treatment; and g) rates of reported side effects. We hypothesized that the mean levels of hemoglobin (primary outcome) would be significantly higher in the Sprinkles arm as compared to the control arm.

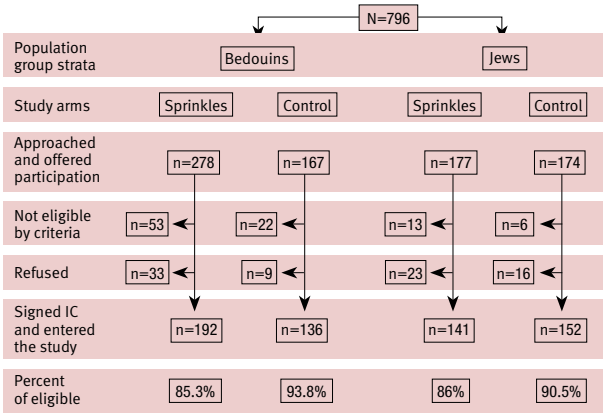
We started by calculating the sample size needed for an individually randomized trial. Using existing data we assumed the hemoglobin concentration at age 12 months would be 11.2 g/dl (standard deviation 1.2) in the control arm, and 12.1 g/dl (SD 1.2) in the Sprinkles arm. Given an alpha level of 5% and power of 80% we needed to obtain data on 36 infants in each of the two arms in each population (Bedouins and Jews). Then, to estimate design effect for a cluster randomized trial using our previous data, we calculated the intra-cluster correlation coefficient as the square of the variation between clusters divided by the sum of squares of variation between the clusters and square of variation within the clusters [16]. Taking an ICC of 0.02, assuming an average of 100 children per MCH clinic (m) and estimated DEF [1+ (m-1)*ICC], the sample size was increased by a factor of 3 from that required for a non-clustered randomized trial. We therefore required a total of 108 infants in each study arm. To take into account an expected dropout rate of 25% we calculated that 135 infants would need to be enrolled in each of the two intervention arms for the Bedouin and the Jewish strata.

STATISTICAL ANALYSIS

Comparisons of background and baseline continuous variables between study arms were examined using ANOVA, while for contingency tables the chi-square test or the Fisher exact tests was used. Baseline data and parameters related to the hematologic, nutritional and growth status of the infants were compared between the Sprinkles and control groups separately for Bedouin and Jewish children. Differences between study arms were considered statistically significant at $P < 0.05$ using two-tailed tests from the analyses, all of which were adjusted for clustering (MCH clinics). Statistical analyses were performed using SPSS-PC 16 and STATA 10.

Hb = hemoglobin
Hct = hematocrit
MCV = mean corpuscular volume
RDW = red blood cell distribution width
TS = transferrin saturation
ICC = intra-cluster correlation coefficient
DEF = design effect

Figure 1. Trial enrollment. Treatment assignments by study arms and population group



IC – Informed Consent

RESULTS

During the enrollment period, parents of 796 infants aged 4 months (445 Bedouin and 351 Jewish) who brought their children for routine follow-up and vaccination to MCH clinics were given an explanation of the study goals and invited to the first study visit at age 6 months (5–7 months) [Figure 1]. Of those, 94 (75 Bedouin and 19 Jewish) infants whose parents arrived for enrollment did not meet the inclusion criteria. Of these, 19 Bedouin and 12 Jewish infants were older than 7.5 months, 44 Bedouin infants lived outside recognized settlements, 5 Bedouin and 7 Jewish infants were exclusively breast fed, and 4 Bedouin and 3 Jewish infants suffered from chronic diseases listed as exclusion criteria. An additional 81 parents (42 Bedouins and 39 Jewish) refused to participate in the study. The final study population comprised 621 eligible infants (293 Jewish and 328 Bedouin) with a mean age of 6.0 ± 1.4 months whose parents agreed to participate, signed an informed consent and completed all enrollment procedures, namely an interview and initial infant blood tests. Overall, agreement to participate was 88.5% (621/702).

SOCIODEMOGRAPHIC AND ANTHROPOMETRIC CHARACTERISTICS

We found that Bedouins and Jews differed in all sociodemographic characteristics [Table 1]: Bedouin parents were younger than Jewish parents and Bedouin fathers and mothers had an average of 3 and 4.5 years of education less than the Jewish parents, respectively. The Bedouins had significantly bigger families, with an average of 2.2 persons per room versus 1.4 in the Jewish families. Bedouin and Jewish infants had a mean birth weight of 3204 ± 490 g without a significant difference

Table 1. Baseline demographic and anthropometric characteristics of study population by population group

Characteristic	Bedouins n=328	Jews n= 293	*P* value
Gender, males, n (%)	206 (51.1%)	211 (54.5)	0.341
Maternal education (yrs)	8.7 ± 4.0	13.2 ± 2.5	< 0.001
Paternal education (yrs)	9.8 ± 3.6	12.8 ± 2.4	< 0.001
Maternal age (yrs)	27.8 ± 6.3	29.3 ± 5.7	0.001
Paternal age (yrs)	31.4 ± 8.0	32.6 ± 6.0	0.022
No. of children in family	4.2 ± 2.9	2.5 ± 1.8	< 0.001
Crowding index	2.2 ± 1.3	1.4 ± 0.6	< 0.001
Birth weight (g)	3203 ± 541	3237 ± 516	0.447
Length for age z score (LAZ)	-0.30 ± 0.1	0.25 ± 1.0	< 0.001
Weight for length z-core (WLZ)	0.13 ± 1.1	-0.05 ± 1.1	0.060

Absolute numbers (n) and percents in parentheses or means ± standard deviations (SD) are presented

Table 2. Hematologic and micronutrient parameters of the study population by population group at enrollment

Variable	Bedouins (n=328)	Jews (n=293)	*P* value
Hemoglobin (g/dl)	10.9 ± 0.9 (10.9)	11.2 ± 1.0 (11.2)	< 0.001
Hematocrit (%)	31.9 ± 2.3 (31.9)	32.8 ± 2.7 (32.9)	< 0.001
Mean corpuscular volume (fl)	73.2 ± 4.3 (73.4)	75.3 ± 3.7 (75.3)	<0.001
Mean corpuscular hemoglobin (pg)	24.7 ± 1.8 (25.0)	25.9 ± 1.6 (25.9)	< 0.001
Mean corpuscular hemoglobin concentration (g/dl)	33.7 ± 1.9 (33.8)	34.2 ± 1.5 (34.2)	< 0.001
Red blood cell distribution width (%)	14.00 ± 1.4 (14.0)	13.7 ± 2.1 (13.45)	0.042
Iron (µg/dl)	44.8 ± 20.9 (41.0)	53.5 ± 27.4 (51.0)	< 0.001
Serum ferritin (µg/L)	41.9 ± 37.0 (35.0)	41.9 ± 34.5 (31.8)	0.127
Tranferrin saturation (%)	13.7 ± 6.8 (12.6)	16.0 ± 7.9 (15.4)	< 0.001
Folic acid (ng/ml)	18.8 ± 4.7 (18.9)	20.6 ± 5.4 (22.6)	< 0.001
Zinc (µg/dl)	128.9 ± 65.7 (112.0)	146.7 ± 79 (124.0)	0.012

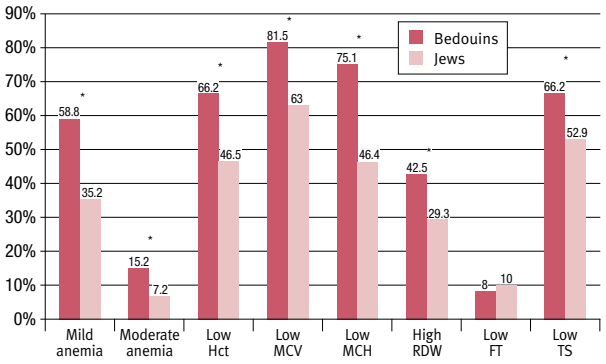
Mean ± standard deviations and medians in parentheses are presented

between them. However, at age 6 months on average (6.0 ± 1.4 month) the anthropometric measurements were higher for Jewish than for Bedouin infants with the most significant difference in measures of stunting (length for age z-score). We also compared the sociodemographic characteristics between the two study arms separately for Bedouins and Jews and found no significant differences between the study arms in either population. Both Bedouin and Jewish study arms had similar sociodemographic characteristics.

HEMATOLOGIC AND MICRONUTRIENT INDICATORS

Anemia and iron deficiency hematologic parameters, including Hb, Hct, MCV, MCH, MCHC and RDW, differed between the

Figure 2. Prevalence of low hematologic indicators among study population by population group



*$P < 0.05$ for difference between Bedouins and Jews
mild anemia (Hb < 11 g/dl)
moderate anemia (Hb < 10 g/dl)
low Hct (< 3 2.9%)
low MCV (< 77 fl)
low MCH (< 26 pg)
low MCHC (< 32 g/dl)
high RDW (> 14%)
low SF (< 15 µg/L)
low TS (< 16%)

Bedouin and the Jewish participants [Table 2]. Bedouin infants also had significantly lower serum concentration of iron, folic acid and zinc. Figure 2 presents the rates of children with low status of hematologic indicators at baseline. Forty percent of 384 Jewish infants had Hb level < 11 mg/dl as compared to 55% of 397 Bedouins ($P < 0.001$). Bedouin infants had higher rates of the following: low levels of Hct (< 32.9%), MCV (< 77 fl), MCH (< 26 pg) and MCHC (< 32 g/dl) and high RDW (> 14%). There was no difference by population in rates of low ferritin level (< 15 µg/L), 9% in both. Only 1.1% of Bedouin and 0.8% of Jewish infants had folic acid deficiency (< 2.7 ng/ml) ($P = 0.54$). Zinc deficiency (< 60 µg/dl) was more frequent in Bedouin (8.8%) than in Jewish children (4.4%).

Comparison of the hematologic and micronutrient indicators by the two study arms showed that while all the examined parameters were similar between the Jewish Sprinkles and control arms, there were minor differences between the Bedouin study arms in mean Hb and Hct levels, which although statistically significant had no clinical significance. The Sprinkles arm had an average Hb concentration of 10.9 ± 0.9 g/dl, while the control arm had a mean level of 10.7 ± 0.9/dl ($P = 0.04$). The Hct level in the Sprinkles arm was 32.2 ± 2.4% vs. 31.6 ± 2.2% in the control arm ($P = 0.02$).

DISCUSSION

Despite current recommendations, the high rates of anemia in infants in these two populations [10,11] – partly due to non-

compliance with those recommendations [10] – called for a new approach to the problem. Multiple micronutrient supplementations seemed to be a viable option for primary prevention due to its reported efficacy in secondary prevention of anemia [9,17-19]. We therefore undertook to examine the efficacy of this treatment versus existing recommendations. If the supplementation, which is easy and inexpensive to provide on a population level, is as effective as shown elsewhere, usually in malnourished populations, it would be justified on clinical, social and economic grounds. In addition to expected effects on anemia and prevention of other micronutrient deficiencies, we hypothesized that the supplementation might even influence hospitalization rates, a high cost item in the health care budget. One of the major differences between Sprinkles efficacy trials elsewhere and in our study is that our infants were not malnourished. The rationale was to test the efficacy of Sprinkles in primary prevention of iron and other micronutrient deficiencies and to evaluate the effect of this supplementation in a well-nourished population of infants and young children. Despite their relatively low socioeconomic status, both the Bedouin and Jewish populations are exposed to western-type facilities including availability of enriched infant formula and foods and free health care services since Israeli law mandates that the whole population has medical insurance. In addition, prenatal care is provided at a nominal fee and vaccinations are given free of charge for those unable to pay. In the Ministry of Health MCH clinics, public health nurses, obstetricians and pediatricians provide up-to-date preventive health care to both pregnant women and infants.

This cluster randomized controlled intervention trial to assess the efficacy of a multiple micronutrient supplementation, Sprinkles, in improving nutritional and health indicators in infants in southern Israel is the first to be conducted in infants with a western-type diet and lifestyle who are not malnourished. In this study stratified cluster randomization was used and the MCH clinics served as randomization units. These sites are appropriate for enrollment as the clinics are neighborhood based and thus deliver services to different population groups at the different locations. MCH clinics were chosen in the populations that have the same socioeconomic status (Bedouins and Jews) and then allocated at random to the two study arms. The cluster randomization method was chosen to reduce cross-over between treatments because of special features of the study populations. This is especially true for the Bedouins who live in big family and tribal units and have extremely close and frequent social contacts, and the topic of the trial would certainly have arisen in their discussions. In addition, we also had to take into account the fact that mothers spend time in waiting rooms before seeing the medical staff. In this setting, the nature of treatments – bottles or sachets – would have become known, and since one treatment might have been perceived as better than the other we were concerned about a high and differential dropout rates. Our inability to provide the treatments in the same physical form resulted in an open trial. Despite the fact that such trials may have substantially reduced statistical efficiency, several attractive features – including increased administrative efficiency, lessened risk of experimental cross-over and likely enhancement of subjects' compliance – are often perceived to outweigh the loss in statistical precision [20].

In contrast to the typical problem of obtaining informed consent in cluster randomized trials [21], we did obtain signed permission from parents of each infant to participate in the study. The participation rate within the eligible population was high (88.5%). The agreement to participate was slightly higher in the control arms (93.8% in Bedouins and 90.5% in Jews) as compared with the Sprinkles arms (85.3% in Bedouins and 86% in Jews). This might be due to parents' concern about administering a new and previously unknown formulation. It also indicates that our concern about refusal and dropout rates in an individually randomized trial was not unfounded.

We examined the success of randomization by comparing the background, sociodemographic and laboratory data between the study arms within strata of populations. The Jewish study arms had similar baseline values for all parameters examined. Among the Bedouins, however, the Sprinkles arm had higher baseline infant hemoglobin and hematocrit levels. While the differences were statistically significant they had no clinical significance.

The study population included term infants who received complementary weaning food with or without breastfeeding. Exclusive breast-fed infants were not enrolled because of the nature of the Sprinkles intervention, which should be added to the weaning food. Since only 17% of Bedouin infants and an even lower percentage of Jewish infants are exclusively breast fed at age 6 months [22], the possibility for bias on this ground was relatively small in this study.

We found very high rates of anemia and iron deficiency indicators in our study population, with statistically significant differences between Bedouin and Jewish infants. Forty percent of the Jewish and more than half (55%) of the Bedouin infants at age 6 months had Hb under 11 g/dl. These findings are supported by high rates of abnormal Hct, MCV, MCH, RDW levels and a high proportion of children with low levels of transferrin saturation. The anemia rates in our study population were higher than in children of other populations with a western lifestyle and usually of similar or higher socioeconomic status. In Estonia, anemia defined as Hb < 10.5 g/dl was found in 9.4% of 9 month old children [23]. In northern Greece, 14% of 8 month old infants were defined as having anemia [24], while only 3% of Norwegian children at age 6 months had Hb < 11 g/dl [25].

Regarding other micronutrient deficiencies, relatively low rates were detected in our study: 1% had folic acid deficiency. A higher percentage of Bedouin (8.8%) as compared to Jewish (4.4%) infants had zinc deficiency.

Despite the similar birth weight of the Bedouin and Jewish infants, the Bedouins had lower mean length-for-age ($P < 0.001$) and lower weight-for-length z-scores ($P = 0.06$) than did Jewish infants at age 6 months. These findings may relate to feeding patterns and/or other cultural and socioeconomic differences between the populations, including maternal nutritional status and spacing of births. These suggestions are supported by the higher fertility rate of the Bedouins compared to the Jewish population [12].

The baseline data indicate that anemia and infant growth issues in the Negev pediatric population are urgent problems that need to be addressed. Our trial examined a novel way to do so via home multi-micronutrient supplementation while taking into account the baseline differences and the clustered nature of the study.

Corresponding author
Dr. N. Bilenko
Dept. of Epidemiology and Health Services Evaluation, Faculty of Health Sciences, Ben-Gurion University of the Negev, P.O. Box 653, Beer Sheva 84105, Israel
Phone: (972-8) 647-7461
Fax: (972-8) 647-7638
email: natalya@bgu.ac.il

References

1. Lutter CK. Iron deficiency in young children in low-income countries and new approaches for its prevention. *J Nutr* 2008; 138: 2523-8.
2. Caballero B. Global patterns of child health: the role of nutrition. *Ann Nutr Metab* 2002; 46 (Suppl 1): 3-7.
3. Bhaskaram P. Micronutrient malnutrition, infection, and immunity: an overview. *Nutr Rev* 2002; 60: S40-5.
4. Lozoff B. Iron deficiency and child development. *Food Nutr Bull* 2007; 28 (4 Suppl): S560-71.
5. Barker DJ. The effect of nutrition of the fetus and neonate on cardiovascular disease in adult life. *Proc Nutr Soc* 1992; 51: 135-44.
6. Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C. Thinness at birth and insulin resistance in adult life. *Diabetologia* 1994; 37: 150-4.
7. Zlotkin SH, Schauer C, Christofides A, Sharieff W, Tondeur MC, Hyder SM. Micronutrient Sprinkles to control childhood anaemia. *PLoS Med* 2005; 2(1): e1.
8. Zlotkin S, Arthur P, Antwi KY, Yeung G. Treatment of anemia with microencapsulated ferrous fumarate plus ascorbic acid supplied as Sprinkles to complementary (weaning) foods. *Am J Clin Nut* 2001 ; 74: 791-5.
9. Hirve S, Bhave S, Bavdekar A, et al. Low dose 'Sprinkles' – an innovative approach to treat iron deficiency anemia in infants and young children. *Indian Pediatr* 2007; 44: 91-100.
10. Bilenko N, Yehiel M, Inbar Y, Gazala E. The association between anemia in infants, and maternal knowledge and adherence with iron supplementation in southern Israel. *IMAJ Isr Med Assoc J* 2007; 9: 521-4.
11. Coles CL, Levy A, Gorodischer R, et al. Subclinical vitamin A deficiency in Israeli-Bedouin toddlers. *Eur J Clin Nutr* 2004; 58: 796-802.
12. Statistical Abstracts of Israel No 572006, Jerusalem.
13. Weitzman D, Shoham-Vardi I, Elbedour K, Belmaker I, Siton Y, Carmi R. Factors affecting the use of prenatal testing for fetal anomalies in a traditional society. *Community Genet* 2000; 3: 60-71.
14. Williams PL, Innis SM. Food frequency questionnaire for assessing infant iron nutrition. *Can J Diet Pract Res* 2005; 66: 176-82.
15. Kuczmarski RJ, Ogden CL, Guo SS, et al. 2000 CDC Growth Charts for the United States: methods and development. *Vital Health Stat* 11 2002; (246): 1-190.
16. Donner A, Birkett N, Buck C. Randomization by cluster. Sample size requirements and analysis. *Am J Epidemiol* 1981; 114: 906-14.
17. Christofides A, Asante KP, Schauer C, Sharieff W, Owusu-Agyei S, Zlotkin S. Multi-micronutrient Sprinkles including a low dose of iron provided as microencapsulated ferrous fumarate improves haematologic indices in anaemic children: a randomized clinical trial. *Matern Child Nutr* 2006; 2: 169-80.
18. Menon P, Ruel MT, Loechl CU, et al. Micronutrient Sprinkles reduce anemia among 9- to 24-mo-old children when delivered through an integrated health and nutrition program in rural Haiti. *J Nutr* 2007; 137: 1023-30.
19. Ip H, Hyder SM, Haseen F, Rahman M, Zlotkin SH. Improved adherence and anaemia cure rates with flexible administration of micronutrient Sprinkles: a new public health approach to anaemia control. *Eur J Clin Nutr* 2009; 63: 165-72.
20. Donner A, Klar N. Pitfalls of and controversies in cluster randomization trials. *Am J Public Health* 2004; 94: 416-22.
21. Cluster randomized trials: Methodological and ethical considerations. MRC clinical trials series© Medical Research Council, November 2002.
22. Belmaker I, Lyandres M, Alsana F, et al. Health status of Bedouin infants and children under age 6 years in established settlements and unrecognized villages in the Negev. ICDC, Ministry of Health, No. of publication 314, December 2008.
23. Vendt N, Grünberg H, Leedo S, Tillmann V, Talvik T. Prevalence and causes of iron deficiency anemias in infants aged 9 to 12 months in Estonia. *Medicina (Kaunas)* 2007; 43: 947-52.
24. Gompakis N, Economou M, Tsantali C, Kouloulias V, Keramida M, Athanasiou-Metaxa M. The effect of dietary habits and socioeconomic status on the prevalence of iron deficiency in children of northern Greece. *Acta Haematol* 2007; 117: 200-4.
25. Hay G, Sandstad B, Whitelaw A, Borch-Iohnsen B. Iron status in a group of Norwegian children aged 6-24 month. *Acta Paediatr* 2004; 93: 592-8.

Appendix 1. Overall study plan and activities by age of participants and location

Age (mos)	Activity	Location/ method
4	**1.** Provide a hand-out study information sheet **2.** Give an invitation to come to an enrollment visit within the next 2 months	MCH clinic*
5.5	**1.** Reminder of date of the enrollment visit	Phone
6	**Enrollment** **1.** Check exclusion and inclusion criteria **2.** Obtain written informed consent **3.** Perform personal interview on: sociodemographic background, breast-feeding and current infant diet (Food Frequency Questionnaire)	MCH clinic
7 -11	**Intervention and follow-up** **1.** Draw venous blood for tests (complete blood count, iron, ferritin, transferrin saturation, zinc, folic acid) **2.** Provide a month's supply of Sprinkles sachets to the Sprinkles arm and iron and vitamins A and D to the control arm **1.** Perform personal interview on: breast-feeding practices, current infant diet (FFQ), use of supplementation as recommended, satisfaction and possible side effects, previous month's morbidity and use of health care services **2.** Provide a month's supply of the supplements as appropriate **3.** Provide a daily diary for recording supplement use for the following month	MCH clinic
12	**1.** Perform a personal interview on breast-feeding practices, current infant diet (FFQ), use of supplementation as recommended, satisfaction and possible side effects, previous month's morbidity and use of health care services **2.** Draw venous blood for tests	MCH clinic
13-17	**1.** Perform a personal monthly interview on: breast-feeding practices, current infant diet (FFQ), previous month's morbidity and use of health services	Phone
18	**1.** Perform a personal interview on infant diet (FFQ) **2.** Draw venous blood tests **3.** Collect the information on documented weight and height from birth until 18 months from the medical records	MCH clinic

MCH clinic = Mother and Child Health clinic