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TO: *Dr. Marge Koblinsky, MotherCare Project, 1616 N. Fort Myer, 11th Floor, Arlington, VA, 22209 USA.*

FROM: *Dr. Michael J. Dibley, Centre for Clinical Epidemiology & Biostatistics, Faculty of Medicine & Health Sciences, University of Newcastle, Room 353, David Maddison Building, Royal Newcastle Hospital, Newcastle, N.S.W. 2300, AUSTRALIA.*

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RE: *Final subcontract report for 5024-116*

Dear Marge:

Please find attached the final sub-contract report for JSI / MotherCare subcontract 5024-116. The report is in the form of a draft of a publication to be submitted to the British Medical Journal. This manuscript is not the final draft but gives a clear indication of the strength of the main findings from the Zibuvita trial. A final manuscript for submission should be completed within the next few weeks. I will keep you informed as the manuscript develops.

Yours sincerely,

Michael J. Dibley.

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Final Subcontract Report

Subcontract No. 5024-116
Between John Snow, Inc. and The University of Newcastle, Australia
to provide services and support under the MotherCare Applied Research Program

ZIBUVITA TRIAL

Prepared by:

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Impact of Vitamin A and Zinc Supplementation on Puerperal Sepsis:

a Randomized Controlled Trial in Rural Indonesia.

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This work was carried out at the Faculty of Medicine, Gadjah Mada University, Yogyakarta Indonesia, and the Centre for Clinical Epidemiology and Biostatistics, Faculty of Medicine & Health Sciences, University of Newcastle, Callaghan Australia.

Reprint requests should be addressed to:.

Running head: Vitamin A and zinc supplementation and maternal sepsis

Introduction

Outline

- Maternal sepsis in developing countries including Indonesia & role in maternal mortality.
- Micronutrient status in pregnancy especially in Indonesia.
- Role of micronutrients in maintaining immune competence
  - vitamin A
  - zinc
- Research question and hypothesis examined in trial

We conducted a double blind, randomized, placebo controlled trial, in pregnant women from a rural Indonesian population with prior evidence of subclinical vitamin A deficiency and a high likelihood of zinc deficiency, which aimed to assess the role of daily low dose vitamin A and zinc supplementation, given from the start of second trimester, in the prevention of puerperal sepsis. The primary study hypotheses were that the incidence of postpartum febrile episodes would be reduced by 45% in the micronutrient supplemented women and that the effects would be greatest in those women receiving both micronutrient supplements.

Study population and methods

Study Protocol

An individually randomized, placebo-controlled, double-masked community based trial was conducted in a rural district of Central Java, Indonesia, to assess the impact of low dose vitamin A and zinc supplementation during pregnancy on maternal puerperal sepsis and other selected maternal and infant pregnancy outcomes. A factorial design was used with four daily supplementation treatment groups: vitamin A; zinc; both zinc and vitamin A; a placebo with no vitamin A or zinc. To examine the main effects of the micronutrient supplementation in the trial (vitamin A versus no vitamin A; zinc versus no zinc), 1,125 mothers were required per group with an equal treatment assignment ratio, to show a 45% reduction in maternal postpartum infections with power $\geq$ 80% (1-β) and 95% confidence (1-α). These sample size estimates were based on an assumed incidence proportion of 5% for puerperal sepsis previously reported in other countries (Koblinsky et al, 1994) This sample size was also expected to have adequate power to examine the hypothesized interaction of the combined nutrient supplementation. However because of social disruptions following the onset of an economic crisis in Indonesia in October 1997 the maternal postpartum infection outcome was only ascertained from the first 627 women enrolled in the trial who delivered during the period 1st April to 31st October 1997.

Study Population

The study was carried out in a district located on the southern coast of Central Java, Indonesia covering an area of 1,034 sq km with a total population of 730,000 and a local crude birth rate of 27.9 per 1,000 population. A special purpose surveillance system was established to monitor the onset of pregnancy in women from 25,000 households located in 8 subdistricts. Women were excluded from intensive pregnancy monitoring if they were not married, or did not have a life-partner, or were pregnant beyond their first trimester, or were currently using a modern hormonal contraceptive or IUD, or were entering the menopause. Informed consent for pregnancy monitoring was obtained from all eligible women. Consenting women were visited at home by female interviewers monthly to record the date of their last menstrual period (LMP) and women whose LMP was delayed more than 5 weeks had their pregnancy status assessed by a urine test in the field. Written informed consent to participate in the trial was obtained from women whose duration of pregnancy was less than 120 days since the LMP. Meetings with village leaders and a program of community education about the trial supplemented the informed consent process. The protocol was reviewed and approved by the Committee on Ethics in Human Biomedical Research of the Faculty of Medicine, Gadjah Mada University.

Randomization and Supplementation

Women who were pregnant for no more than 120 days were randomized to a treatment group following informed consent and enrolment in the trial. Treatments were assigned in a 1:1:1:1 ratio in blocks of 12 based on a list of treatment numbers derived from a pseudo-random number generator in SAS (SAS, 1998). The supplements were coded with the treatment numbers and women were assigned a treatment number from a master list by the field coordinator in sequence based on the date they consented to participate in the trial. The treatment allocation sequence was prepared and held at the University of Newcastle, a site remote from the trial. All investigators, field and laboratory staff and participants were blinded to the treatment code until all field data

were collected and preliminary data analysis by coded groups was completed. A survey of a sample of field workers and their supervisors revealed that they were unable to identify which treatments the study participants were receiving.

The supplements were packaged in plastic strips in identical opaque pink capsules and contained either 2,400 retinol equivalents (RE) of vitamin A as retinyl palmitate; or 20 mg of zinc sulphate; or the same dose of both vitamin A and zinc sulphate; or a placebo with no vitamin A or zinc. The dose of vitamin A was designed to be sufficient to ensure that vitamin A status could be restored and maintained during pregnancy in a vitamin A deficient population. The daily intake of vitamin A provided was approximately 3 times the required dietary allowance (RDA) for pregnant women in the United States of 800 RE/day and about twice the average observed intake of pregnant women in the United States of about 1,200 RE/day (National Academy of Sciences, 1990). However, this dose was below the generally accepted safe limit of vitamin A intake in pregnancy of 3,000 RE/day over extended periods of time (Underwood 1994). The dose of zinc was designed to ensure an intake equivalent to the FAO/WHO/ILEA normative requirements for zinc in pregnancy (Gibson, 1994). All capsules also contained 2mg dl-α-tocopherol as an antioxidant and 350 mg of soybean oil, 20 mg of bees wax and 8 mg of lecithin as capsule filler.

Data collection procedures

Distribution of interventions and monitoring compliance: Supplements were distributed to women enrolled in the trial at home by a fieldworker who also monitored their compliance with the trial treatment and collected information about pregnancy morbidity. Initially these visits took place every fortnight but were reduced to a monthly frequency once the woman was familiar with the supplementation program and had demonstrated a high level of compliance. At each visit plastic strips with sufficient supplements for 2 weeks or 1 month were distributed. Any unused capsules were returned to the field worker at the following home visit and were counted and recorded to monitor compliance. Women who experienced difficulties with the study protocol were visited by specially trained field staff for counselling. The women's intended birth attendant was visited during this period to explain the trial and seek their support during the delivery and postpartum period.

Examinations and monitoring of morbidity during pregnancy: At enrollment the women were physically examined and blood collected for assessment of micro nutrient status. Thereafter they were visited monthly by a fieldworker who recorded symptoms of pregnancy morbidity and use of health care services. Weight and height were also measured at enrollment by trained anthropometrists whose measurement techniques had been standardized. Weight was measured to 0.1 kg with automatically calibrated electronic scales (Seca Model 835, CMS Ltd., London, United Kingdom) and height was measured to 0.1 cm with a portable stadiometer (CMS Ltd., London, United Kingdom).

Detection of onset of labour: A team of field workers visited daily the villages and birth attendants of all women who were within 1 month of their expected delivery date. Once a trial participant had entered labour, this team alerted the field nurses who aimed to visit the women within 24 hours of delivery.

Detection of postpartum infections: Following delivery, trained field workers visited all trial participants every day from day 2 to day 10 postpartum and on days 12 and 14 postpartum to record morbidity symptoms, and to measure body temperature using an audible electronic

thermometer (Model SB-70, Nipro Company, Tokyo, Japan). The follow up schedule allowed for a maximum of 11 days of postpartum observations. *Reported feverishness* was based on the daily interviews with women during the postpartum period and needed to be reported twice to establish a feverishness event. *Puerperal fever* was defined as a body temperature $\geq 37.5°C$ occurring on any occasion during the first 14 days postpartum, exclusive of the first 24 hours, and taken by mouth daily using a standard technique. *Puerperal sepsis* was defined as a body temperature $\geq 38°C$ occurring on any occasion during the first 14 days postpartum, exclusive of the first 24 hours, and taken by mouth daily using a standard technique. This later definition is a modification of the standard clinical definition of puerperal sepsis which requires an elevated body temperature $\geq 38°C$ on any 2 of the first 10 days postpartum, exclusive of the first 24 hours, but is based on temperature measurements being taken at least 4 times day (Cunningham et al. 1997). This frequency of measurement of body temperature was not possible in a community based trial.

Laboratory procedures

Collection of blood specimens: A blood sample was collected by specially trained nurses from all women on entry to the trial which was usually at the end of the first trimester or start of the second trimester. The blood collection and laboratory equipment were free of contamination with zinc and all specimens were centrifuged in a field laboratory within 2 hours of collection, stored at -4°C, protected from direct light and dispatched on ice the same day to the biochemistry laboratory at the University of Gadjah Mada.

Vitamin A: Vitamin A status was assessed using serum retinol concentrations. The serum specimens were processed and stored according to IVACG guidelines and the retinol levels were assayed using a standard High Pressure Liquid Chromatography method (IVACG 1982) already established in the laboratory. The within-assay coefficient of variation for the retinol analyses was less than 5%. Low serum retinol in women was defined as <30 μg/dL. In the first National Health and Nutrition Examination Survey (Pilch, 1987) in the United States, < 4% of women 18-44 years of age had serum retinol levels < 30 μg/dL, however the proportion of women below this cutoff should be higher during pregnancy but no reference data are available. Serum retinol values > 100 μg/dL were excluded from analyses.

Zinc: A thermal neutron activation method was used to assess the serum concentrations of zinc (Woittiez, 1984).

Haemoglobin: Haemoglobin levels were measured using the Hemocue, a portable blood haemoglobin photometer (Hemocue AB., Ängelholm, Sweden). Anaemia was defined as a mean haemoglobin concentration of <11 g/dl.

Data management and statistical analysis

Field and laboratory data were collected on precoded forms that were subject to a system of editing before data entry. The dSURVEY (Corner 1989) software was used for data entry and data cleaning. Data analyses were carried out on microcomputers using SYSTAT (SPSS 1998) and SAS (SAS 1996) statistical software. Baseline differences between treatment groups were tested by χ^2 test for general association or t-tests for normally distributed continuous variables. Differences between treatment groups were judged to be significant at $P < 0.05$. Compliance was assessed by estimating the proportion of the distributed capsules that were consumed based on pill counts. A geometric mean was used to calculate the average percentage of capsules consumed by trial participants. Postpartum febrile events were evaluated on an intention to treat basis with all women assigned to each supplementation group included in analyses irrespective of their level of compliance with the trial treatment. Incidence proportions (Rothman & Greenland 1998) for

postpartum febrile events were calculated for each treatment group. Relative risks with 95% confidence intervals were used to assess treatment effects with either the placebo group as the reference, or the treatment groups not receiving the micronnutrient supplement being examined as the reference.

Results

A total of 1 383 women with their last menstrual period between 24/6/96 and 30/4/97 were eligible for the trial and formed the cohort of women from whom the initial 660 women whose pregnancies ended between the 1st April and 31st October 1997 were derived (Figure 1). A total of 1 008 of the eligible women were randomized to received a trial treatment. In all 375 (27%) of the eligible women were not randomized because they either did not consent (2.7%) or were not available to provide consent when field staff visited their homes (17.3%) or emigrated from the study area (7%). A total of 343 women, or 30 to 35% of each treatment group, had not delivered by 31st of October 1997 and were not included in the analyses. Also a total of 31 women or 1.6% to 4.5% of each treatment group terminated their pregnancies because of spontaneous abortion or preterm delivery prior to 1st April 1997 and were also not included in the analyses. Across all treatment groups outcome data was not available for 32 women who withdrew from the trial, 56 women who had spontaneous abortions and 12 women who had stillbirths during the period 1st April and 31st October 1997. A total of 41 women had incomplete postpartum surveillance data but because their delivery occurred in the period that defined the study cohort they were included in denominators for estimating incidence proportions.

At entry to the trial the treatment groups were comparable in demographic characteristics, nutritional status, reproductive history and socioeconomic characteristics. Small differences between treatment groups were noted for household expenditure on food, electricity in the house and possession of TV sets. The median household expenditure on food was lower for the vitamin A, and vitamin A and zinc treatment groups in comparison to the other groups. Electricity was found in a lower percentage of households in the zinc treatment group than in the other groups. Possession of TV sets was lower in the vitamin A alone and zinc alone treatment groups (Table 1). The distribution of place of delivery and primary birth attendant for the trial pregnancy was similar for all treatment groups. Overall 78% of women delivered at home and 58% were attended by midwives and 33% by traditional birth attendants.

The median number of home visits during pregnancy, supplement capsules distributed and days of follow up after delivery was similar in all treatment groups. The mean number of days of follow up postpartum was slightly lower in the placebo group although the total number of observed days postpartum was similar for all treatment groups. Overall the percentage of distributed capsules consumed was high although it was slightly lower in the placebo group. Twenty five percent of all the trial participants consumed 65% or less of the distributed capsules (Table2). (Will add information about the response of serum retinol and zinc to treatment in the subsample of women for whom multiple blood samples were collected. This information should confirm the high compliance with treatment).

The proportion of women reporting at least 2 days of feverishness during the postpartum period was 12% in the placebo group. In contrast only 5.2% of women receiving vitamin A supplementation reported at least 2 days of feverishness yielding a relative risk of 0.46 (0.25 to 0.87) in comparison to the treatment groups not receiving vitamin A. The zinc supplementation did not have an impact on reported feverishness nor was there evidence of an interaction between vitamin A and zinc (Table 3).

Puerperal pyrexia, with body temperature > 37.5°C on at least one day in the postpartum period, was observed in 15.5% of the women in the placebo group. The proportion of women with puerperal pyrexia was lower (9.6%) in the vitamin A treated groups however the 46% reduction was not statistically significant. There was a 16% reduction in the proportion of women with puerperal pyrexia in the zinc treated group but this also was not statistically significant (Table 4). Pyrexia was reported on two or more days in 6.7% of the non-vitamin A treated women but this fell to 2.8% in the vitamin A treated women yielding a relative risk of 0.42 (0.18 to 0.98).

Puerperal sepsis, with body temperature > 38.0°C on at least one day in the postpartum period, was observed in 11.3% of the women in the placebo group. The proportion of women with puerperal sepsis was much lower (2.8%) in the vitamin A treated groups and this 70% reduction was signficant. An even greater reduction (79%) was observed in the women treated with vitamin A alone in comparison to the placebo group. In the zinc treated groups there was a 30% reduction in the proportion of women with puerperal sepsis although it was not significant. A comparison the zinc alone versus the zinc and vitamin A treated groups reveals the greater impact of vitamin A on puerperal sepsis. There was no interaction of effect on puerperal sepsis between the micronutrients (Table 5).

Figure 1. Flow of participants through various stages of the trial with details of randomization, withdrawals and follow up.

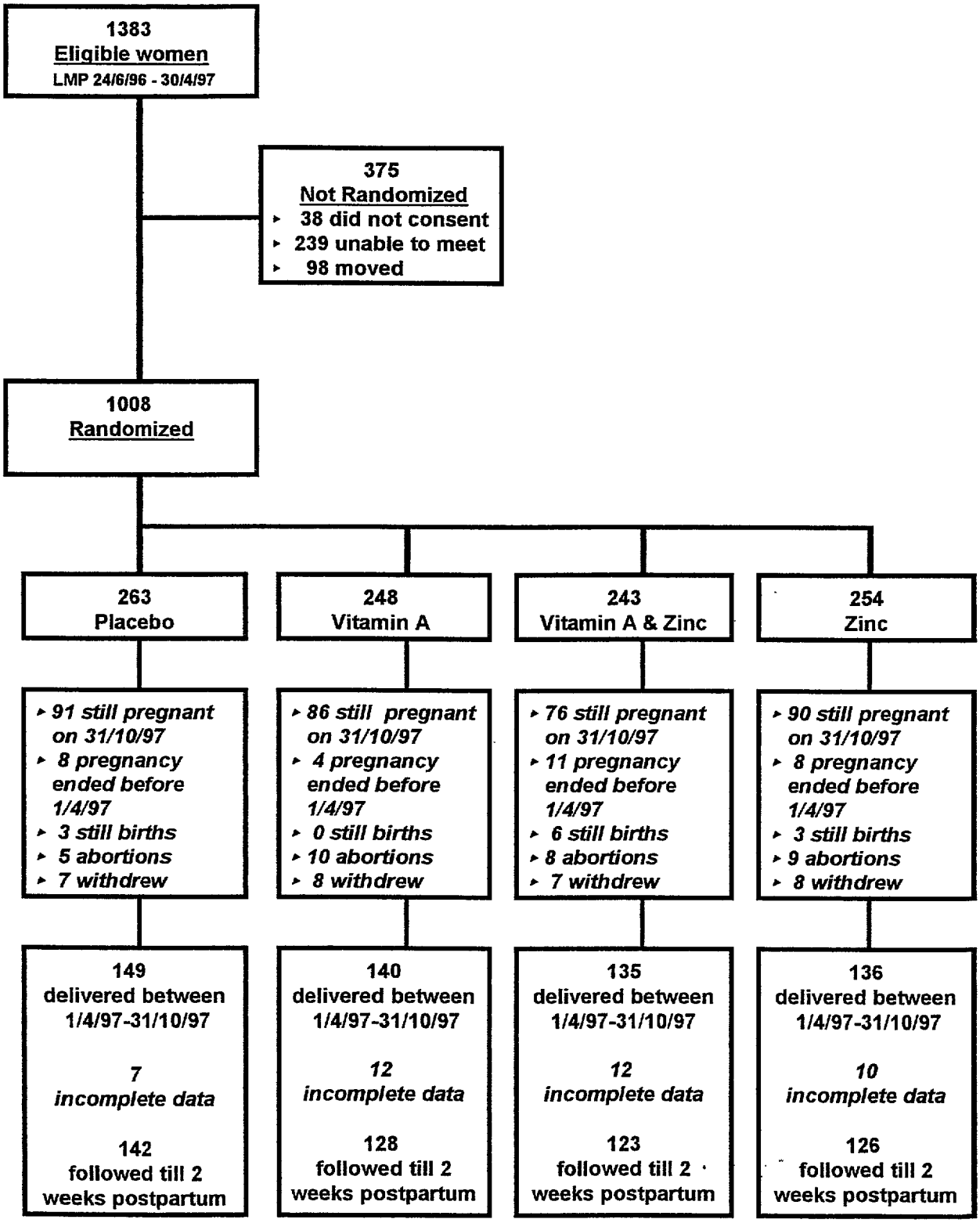

Table 1. Characteristics of study participants on entry to trial by treatment groups. Values are numbers (percentages) unless otherwise stated.

Characteristic	Treatment groups			
	Placebo	Vitamin A alone	Zinc & Vitamin A	Zinc alone
Demographic characteristics				
Age				
< 20 years	3 (2.1)	12 (9.4)	9 (7.3)	4 (3.2)
20-29 years	78 (54.9)	71 (55.5)	68 (55.3)	65 (51.6)
≥ 30 years	61 (43.0)	45 (35.2)	46 (37.4)	57 (45.2)
Mean Age in years ± SD	28.4 ± 4.03	27.6 ± 4.99	27.8 ± 4.68	28.4 ± 4.86
Education of mother				
Total number of family members	4.1 ± 1.36	3.8 ± 1.27	4.0 ± 1.44	3.9 ± 1.18
Nutritional status				
Maternal height (cms)				
MUAC (mm)				
Serum retinol (μmol/L)				
Serum zinc				
Hemoglobin (g/dl)	12.3 ± 1.53	12.3 ± 1.79	12.1 ± 1.68	12.4 ± 1.45
Reproductive History				
Mean Parity ± SD	2.6 ± 1.11	2.5 ± 1.14	2.6 ± 1.10	2.5 ± 1.09
No previous deliveries	22 (15.5)	23 (18.0)	16 (13.0)	22 (17.5)
Previous abortions	23 (16.3)	19 (15.1)	17 (13.8)	22 (17.6)
Birth attendant for trial pregnancy				
Family member	7 (4.9)	10 (7.8)	2 (1.6)	4 (3.2)
Traditional birth attendant	46 (32.4)	45 (35.2)	36 (29.3)	42 (33.3)
Midwife	80 (56.3)	67 (52.3)	78 (63.4)	74 (58.7)
Doctor	9 (6.3)	6 (4.7)	7 (5.7)	6 (4.8)
Place of birth for trial pregnancy				
Hospital	17 (12.0)	16 (12.5)	22 (17.9)	22 (17.5)
Maternity clinic	10 (7.0)	9 (7.0)	6 (4.9)	12 (9.5)
Home	115 (81.0)	103 (80.5)	95 (77.2)	92 (73.0)

Table 2. Follow up during pregnancy and postpartum period and compliance with trial supplementation by treatment groups.

Characteristic	Treatment groups			
	Placebo	Vitamin A alone	Zinc & Vitamin A	Zinc alone
Pregnancy follow up				
Home visits during pregnancy				
Median no. of home visits (25th , 75th percentile)	9 (8, 9)	9 (8, 9)	9 (8, 9)	9 (8, 9)
Mean ± SD	8.4 ± 1.33	8.6 ± 1.11	8.3 ± 1.28	8.5 ± 1.19
Number of capsules distributed				
Median no. of capsules (25th , 75th percentile)	210 (180, 221)	210 (195, 221)	210 (180, 210)	210 (180, 225)
Mean ± SD	200 ± 35.8	203 ± 31.4	197 ± 36.0	200 ± 37.9
Postpartum follow up				
Median no. of days (25th , 75th percentile)	11 (9,11)	11 (11,11)	11 (10, 11)	11 (10, 11)
Mean number of days ± SD	8.9 ± 3.75	9.8 ± 2.88	9.5 ± 3.35	9.2 ± 3.61
Total number of observed days	1066	1066	999	1040
Compliance with treatment				
Median of 'percentage of distributed capsules consumed' (25th , 75th percentile)	76.8 (64.3, 85.0)	78.1 (64.9, 87.2)	77.6 (64.8, 85.2)	79.8 (69.6, 86.2)
Mean* of 'percentage of distributed capsules consumed'	70.7	72.8	71.3	72.1

* Geometric mean

Table 3. Proportion of women reporting feverishness on at least two days during 14 days postpartum but excluding the first day, by treatment groups.

Treatment group	Number of pregnancies	Number of cases	Reported feverishness %	Rate ratio *(95% CI)*
Main effects				
No Vitamin A	268	30	11.2%	1.00
Vitamin A	251	13	5.2%	0.46 *(0.25-0.87)*
No Zinc	270	22	8.2%	1.00
Zinc	249	21	8.4%	1.04 *(0.58-1.84)*
All treatment groups				
Placebo	142	17	12.0%	1.00
Vitamin A alone	128	5	3.9%	0.33 *(0.12-0.86)*
Vitamin A & Zinc	123	8	6.5%	0.54 *(0.24-1.22)*
Zinc alone	126	13	10.3%	0.86 *(0.44-1.70)*

Table 4. Proportion of women with body temperature >37.5°C on at least one day during 14 days postpartum but excluding the first day, by treatment groups.

Treatment group	Number of pregnancies	Number of cases	Percent with Fever	Rate ratio *(95% CI)*
Main effects				
No Vitamin A	268	40	14.9%	1.00
Vitamin A	251	24	9.6%	0.64 *(0.40-1.03)*
No Zinc	270	36	13.3%	1.00
Zinc	249	28	11.3%	0.84 *(0.50-1.34)*
All treatment groups				
Placebo	142	22	15.5%	1.00
Vitamin A alone	128	14	10.9%	0.71 *(0.38-1.32)*
Vitamin A & Zinc	123	10	8.1%	0.52 *(0.26-1.06)*
Zinc alone	126	18	14.3%	0.92 *(0.53-1.64)*

Table 5. Proportion of women with body temperature >38.0°C on at least one day during 14 days postpartum but excluding the first day, by treatment groups.

Treatment group	Number of pregnancies	Number of cases	Percent with fever	Rate ratio *(95% CI)*
Main effects				
No Vitamin A	268	25	9.3%	1.00
Vitamin A	251	7	2.8%	0.30 *(0.13-0.68)*
No Zinc	270	19	7.0%	1.00
Zinc	249	13	5.2%	0.70 *(0.37-1.47)*
All treatment groups				
Placebo	142	16	11.3%	1.00
Vitamin A alone	128	3	2.3%	0.21 *(0.06-0.70)*
Vitamin A & Zinc	123	4	3.3%	0.29 *(0.10-0.84)*
Zinc alone	126	9	7.1%	0.63 *(0.29-1.38)*

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