Supplementary Table. Effects of supplementation with multiple micronutrients on fertility mechanisms and outcomes

Study (country)	Study characteristics		Number of patients, N		Mean age ± SD (range), y	Supplements/control groups		Results	Author conclusions
	Design	Duration	Multiple micronu-trients (MMN)	Control group(s)		Investigational product: folate [µg] vs. Control	Taken PC		
Effect in healthy women									
Czeizel et al 1996[1] (Hungary)	Double-blind RCT in women planning pregnancy	~4 mo	3953	3952	26.9±3.4	Elevit (Bayer): 800 vs. Trace element supplement (Cu, Mn, Zn, vit. C)	Yes, and T1	• Significantly higher number of confirmed pregnancies with MMN (2553, 64.6%) vs. trace elements (2466, 62.3%) (excl. non-supplemented women) • Time to conception was shorter with MMN (3.8 menstrual cycles) vs. trace elements (4.0 cycles), suggesting a 5% increase in fertility	• Preconceptional MMN supplementation causes a slight but significant increase in fertility due to the shorter achievement of conception
Cueto et al 2015[2] (Denmark)	Prospective cohort study in women planning pregnancy	Varied duration	2560	1335	28.6 (supplement group)	FA and/or MMN (either used alone or together; type not specified) vs. Non-use	Yes	• Increased fecundability with supplement use • Stronger association among women with irregular cycles and those with either short or long cycle length	• Longer duration of supplementation (≥1 year) did not increase fecundability
Chavarro et al 2008[3] (USA)	Prospective cohort study in women planning pregnancy	8 years FO	10451	8104	32.5 (supplement group)	MMN (type not specified) vs. Non-use	Yes	• Inverse association between frequency of MMN use and risk of ovulatory infertility • RR of ovulatory infertility with MMN use compared with non-use was 0.88 for women consuming ≤2 tablets/week, 0.69 for women consuming 3-5 tablets/week, 0.59 for women consuming ≥6 tablets/week	• Folic acid appeared to explain part of the association between MMN use and risk of ovulatory infertility • Regular use of MMN supplements may decrease the risk of ovulatory infertility

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Effect in women undergoing infertility treatment									
Restoring micronutrient levels									
La Vecchia[4] (Italy)	Cross-sectional study in women undergoing IVF; analysis of plasma levels of vitamins or microelements	—	125	144	37.1±3.5	FA supplement (type not specified) vs. Non-use	Yes	• Users significantly more likely to have adequate levels of serum folate, RBC folate and HCY compared with non-users • Only a minority of participants reached adequate levels of RBC folate despite long-term FA supplementation: proportion of replete women (>400 ng/ml): 25% and 23% in participants women using FA supplementation for >12 and <12 months, respectively • In the absence of FA supplementation, the proportion of replete women was nearly negligible	• Folate levels largely inadequate in women undergoing IVF, but higher in women using supplements vs. non-users • There is a need to promote recommendations for FA supplementation among women attending infertility clinics
Özkaya et al 2011[5] (Turkey)	PBO-controlled RCT in IVF patients	45 d	26	43	28.8±3.2 (22-43)	Megadyn Pronatal Film Tablet (Mecom Inc): 800 vs. Placebo	Yes	• IVF vs. controls: ↓selenium and zinc in FF and serum; ↓copper in serum; ↑ iron in FF and serum • Supplementation vs. untreated IVF: ↑ copper, zinc, selenium in FF and serum; ↓iron in FF; calcium, magnesium levels unaffected	• Copper, zinc, and selenium in serum and FF were lower in women undergoing IVF • MMN supplementation in serum and FF of such women normalized trace element levels

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Sun et al 2013[6] (China)	PBO-controlled RCT in IVF patients	60 d	60	55	28.8	Elevit (Bayer): 800 vs. Placebo	Yes	• IVF vs. controls: ↓copper and zinc in serum • Supplementation vs. untreated IVF: ↑copper, zinc and manganese in serum; ↑copper and zinc in FF; ↓iron in FF; iron and calcium in serum unaffected	• Taking MMN might normalize trace element levels in the serum and FF of women undergoing IVF
Impact on antioxidant defenses, oxidative stress and fertility									
Özkaya et al 2010[7] (Turkey)	PBO-controlled RCT in IVF patients	45 d	26	43	28.8±3.2 (22-43)	Megadyn Pronatal Film Tablet (Mecom Inc): 800 vs. Placebo	Yes	• IVF: significantly ↓antioxidant vitamins (C, GSH-Px) and ↑LP in FF and serum • Supplementation vs. untreated IVF: significantly ↑GSH, vit. C & E in serum, ↑GSH-Px and vit. C in FF; significantly ↓LP in FF and serum	• MMN supplementation in women undergoing IVF may strengthen the antioxidant defense system by decreasing oxidative stress
Luddi et al 2016[8] (Italy)	Preliminary study in IVF patients (no MMN in first COS cycle, MMN in second COS cycle)	MMN used for 3 mo before and through-out second COS cycle	18	—	40.3±1.2	Elevit (Bayer): 800 vs. Non-use in first COS cycle	Yes	• MMN (used in the second COS cycle only) protected the follicular microenvironment and serum proteins from oxidative damage • Significant increase in mean number of good quality oocytes after MMN usage • In the treated cycle, a total of 3 ongoing pregnancies (pregnancy rate=17.7%) was registered	• MMN supplementation may decrease oxidative stress both in serum and follicular fluid proteins and is positively associated with oocyte quality in women undergoing IVF

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Nouri et al 2017 [9]	Pilot, non-blind RCT in women undergoing IVF	28-56 d prior to COS	50	50	Median 37.1 (IQR 33.6-40.2)	PROfertil® female (Lenus Pharma GesmbH): 800 vs. FA: 400	Yes	• Median fertilization rate significantly higher with MMN (66.7%) vs. FA use (42.9%) • Significantly more MMN patients with at least one high quality embryo (29%) vs. FA patients (18%)	• Use of MMN supplementation for a minimum of 28 d is beneficial in terms of fertilization rate and embryo quality
Youssef et al 2015[10]	RCT in women with unexplained infertility	58 d	112	106	30.9±5.7 (MMN group)	Octatron® (Nerhadou International) + FA: 2500 vs. FA: 2500	Yes, and T1	• No significant differences between groups, including number of mature oocytes and clinical pregnancy rate	• Oral antioxidants from MMN did not improve oocyte quality and pregnancy rates in women with unexplained infertility undergoing IVF/ICSI treatment
Impact on the time to pregnancy and chances of becoming pregnant									
Ruder et al 2014[11]	Secondary data analysis of RCT in women with unexplained infertility	—	368	—	33.1	MMN (type not specified)	Yes	TTP was shorter among women: • with BMI <25 kg/m² with increasing vitamin C • with BMI ≥25 kg/m² with increasing β-carotene • <35 y with increasing β-carotene and vitamin C • ≥35 y with increasing vitamin E	• Increased intakes of β-carotene, vitamin C, and vitamin E were associated with shorter TTP, but the effect of these antioxidant nutrients varied with BMI and age • The results are consistent with the hypothesis that increased antioxidant intake is positively associated with female fertility
Westphal et al 2004/2006[12,13]	Double-blind, PBO-controlled RCT in women struggling to conceive	3 mo	53	40	35.4 (MMN group)	FertilityBlend™ (Daily Wellness Co.) vs. Placebo	Yes	• Significant increase in average number of days in cycle with basal temperature >37°C during the luteal phase with MMN vs. baseline	• MMN supplementation may provide an attractive alternative or complement to conventional fertility therapy

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								• Increase in mean midluteal phase progesterone level with MMN vs. baseline, significant in women with the lowest progesterone levels at baseline • Short and long cycles were normalized with MMN • No notable changes at all with PBO • After 3 mo, 26% of MMN women pregnant vs. 10% PBO women; three more MMN women conceived after 6 mo	• Nutritional supplementation may play an important role in optimizing fertility health
Agrawal et al 2012[14]	Pilot, double-blind RCT in women with unexplained infertility	3-6 mo	30	28	32.2 (MMN group)	Pregnacare Conception (Vitabiotics) + FA: 400 + 400 vs. FA: 400	Yes	Compared with FA alone, women using MMN supplementation had: • significantly fewer attempts to achieve pregnancy • a significantly higher cumulative clinical pregnancy rate (66.7% vs. 39.3%) • a significantly higher ongoing pregnancy rate (60.0% vs. 25.0%)	• Study suggests that women who use adjuvant MMN supplementation during ovulation induction have a higher chance of pregnancy compared with women on FA alone • Women susceptible to micronutrient deficiencies should receive micronutrient supplements to optimize their reproductive health

BMI, body mass index; COS, controlled ovarian stimulation; d, days; FA, folic acid; FF, follicular fluid; FO, follow-up; GSH-Px, glutathione peroxidase; HCY, homocysteine; IQR, interquartile range; IVF-ET, in vitro fertilization-embryo transfer; LP, lipid peroxidation; MMN, multiple micronutrients; mo, months; PBO, placebo; PC, pre-conception; PP, post-partum; RBC, red blood cells; RCT, randomized, controlled trial; ROS, reactive oxygen species; SD, standard deviation; T1, first trimester; TTP, time to pregnancy; wks, weeks; y, years.

Supplementary references

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