The impact of oral multinutrient supplementation on in vitro fertilisation or intracytoplasmic sperm injection outcomes: A prospective controlled study

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ABSTRACT

Introduction: Micronutrients influence female fertility, thus adequate levels are important for oocyte quality, maturation, fertilisation and implantation. This study prospectively evaluated the impact of oral multinutrient supplementation on fertility outcomes in In vitro fertilisation or Intracytoplasmic sperm injection (IVF/ICSI).

Materials and Methods: This was a pilot study of N=50 women, who were planning for IVF treatment in University Malaya Medical Centre, Kuala Lumpur, Malaysia from July to December 2023. Women without prior nutritional treatment were consented and assigned to either the multinutrient supplementation (Omega 3, coenzyme Q10, folic acid, selenium, vitamin E, catechins) as the study group or 5mg folic acid daily as control group for at least a month prior to their IVF treatment. All women were treated using an antagonist protocol and ovarian stimulation was started with 200 –300IU of urinary HMG and or recombinant FSH. Antagonists (Ganirelix) commenced when the leading follicle reached a diameter of 11 mm. Triggering with hCG or GnRH agonist when at least 3 follicles of 17 mm in diameter were achieved. Oocyte retrieval was performed 36th hour after trigger. Conventional IVF/ICSI was used for fertilisation. All parameters recorded and analysed using SPSS.

Results: The mean age (36.44 ± 3.33 vs 35.32 ± 3.47 years) and body mass index (25.28 ± 4.12 vs 24.80 ± 4.36 kg/m2) of women in multinutrient supplementation group was similar to control group. The Follicular Output Rate (FORT) in women on multinutrient supplementation showed a trend towards benefit compared to control group, although it is not statistically significant (68.12 ± 19.47 vs 64.91 ± 20.06, p=0.493). The mean number of MII oocytes retrieved from mature follicles and number of good quality embryo on day 3 after fertilisation were not statistically significant between the two groups (6.65 ±3.84 vs 6.09 ± 3.01, p=0.626 and 4.00 ± 3.10 vs 3.45 ± 2.30, p=0.549, respectively). In addition, there were no differences in endometrial thickness before embryo transfer in both groups (10.35 ± 1.32mm vs 10.36 ± 2.04mm, p=0.320). However, the total dose of follicle stimulating hormone and duration of controlled ovarian stimulation were lower in the study group compared to control group (2410 ± 656.82 IU vs 2706.82 ± 536.15 IU, p= 0.119 and 8.90 ± 2.13 days vs 9.68 ± 1.29 days, p=0.164, respectively).

Conclusion: A multinutrient supplementation given for a minimum of 28 days, may have a positive effect on FORT and lower use of gonadotropin. More and larger sample research is warranted to prove this effect.

KEYWORDS:
Multinutrient, Female fertility, Follicular Output Rate (FORT), In vitro Fertilisation, Intracytoplasmic Sperm Injection, Endometriosis

INTRODUCTION

Subfertility among Malaysian has recently gained the national authority attention with the decline of total fertility rate reported to came down to below replacement level.[1] Similar to many countries, many women delay conception to acquire higher education, pursuing a career, achieving financial stability and plan for late marriage.[1-3] Due to these circumstances, many women were aged with reduced ovarian reserve when they decide to conceive.[4-6] Approximately 70% of couples have no identified cause which diverted the solution to restoring potentially modifiable risk factors such as micronutrient status that may have an impact on female fertility.[7-8] Essential vitamins and minerals have important roles in the physiological processes and therefore adequate levels are important for oocyte quality, maturation, fertilisation and implantation whereas antioxidants are vital to reduce oxidative stress, a process known to impair fertility.[9] Lower recommended micronutrients levels have been reported in sub fertile women.[10-11] A similar scenario has been found in a proportion of women of childbearing age in general, some of whom may be struggling to conceive.

Recent scientific discussions recognised the important role of micronutrients in fertility that should be addressed. Micronutrients are essential vitamins and minerals that are required in small quantities as dietary components. Even though these micronutrients do not provide energy to the human body, they are essential for catabolic and anabolic processes and need to be supplied externally.[2] The importance of good nutrition has already been established during pregnancy, influencing embryonic and foetal development, and thus pregnancy outcomes. Overall, supplementation has a small but beneficial effect on fertility in healthy and infertile women, including a shorter time to

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pregnancy and an increased chance of becoming pregnant.[8] Much less is known about the influence of micronutrient status on female fertility, and human studies are scarce.[12]

This study was conducted to look into the impact of oral multinutrient supplementation on fertility outcome in In vitro fertilisation or Intracytoplasmic sperm injection. The main outcome of this study is to compare the follicular output rate (FORT) between oral multinutrient and folic acid in women undergoing IVF/ICSI.

MATERIALS AND METHODS

This was a prospective pilot study of 50 women, who were planned for IVF/ICSI treatment at the university-based fertility centre in Malaysia from July to December 2023. As a pilot study, power calculation was not required. Women without prior nutritional treatment were consented and assigned to either the multinutrient supplementation as the study group or folic acid daily as control group. Participant information sheets were given to all potential recruits and any inquiries by the participants were answered by the recruiting care provider. The first 25 consecutive women who attended the clinic during the period of study were enrolled into the study group and the subsequent 25 consecutive women into the control group. Women between 19 to 41 years of age were included, whereas those with body mass index more than 35 kg/m^2, women who had taken any multinutrient supplementation preparation other than folic acid alone for the last 3 months and required husband's testicular sperm for treatment were excluded. All recruited women were seen in the clinic as per protocol, routine blood investigations were done and reviewed. At the beginning of the menstrual cycle, transvaginal ultrasound was performed by a sonographer to look for any pelvic pathology, endometrial thickness, bilateral ovaries and total antral follicles count. Investigator gave a phone call to enquire regarding the compliance and any side effects after three weeks of medication initiation. After completion of a minimum of 28 days of the medication, women were advised to call the clinic on her first day of following immediate menstruation to arrange for baseline scan as routine preparation of ovarian hyperstimulation as per local protocol. All patients were given a patient information sheet and signed a written informed consent. The study was approved by the Medical Research Ethics Committee of the University Malaya Medical Centre, Kuala Lumpur, Malaysia (MREC ID No: 2023726-12708, finally approved on December 10, 2023).

Women were treated with a multinutrient preparation (Fortelle + Omega-3, BSV Bioscience Malaysia) consisting of the daily dosage of one soft capsule and one tablet, both taken for at least 28 days prior to ovarian hyperstimulation. The soft capsule contains omega-3 fatty acid (eicosapentaenoic acid (EPA) 119mg and docosahexaenoic acid (DHA) 316mg) and the tablet contains: coenzyme Q10 30mg, vitamin E 45IU, folic acid 800mcg, selenium 76mcg, and catechins 4mg- green tea leaf extract.

While the control group were given one tablet of folic acid 5mg to be taken for at least 28 days prior to treatment. From the literature review, the effects of multinutrient on oocyte recruitment which observed in the follicular phase after antioxidant intake for at least one cycle length. Hence, a minimum of 1 month intake of multinutrient was warranted in our study before a possible effect can be achieved.[2,4]

All women from both groups were using an antagonist protocol for controlled ovarian stimulation, stimulation typically began on day 2 to 4 of menses and ovarian stimulation was started with 200 –300IU of recombinant follicle stimulating hormone (rFSH) alone or Human Menopausal Gonadotropin (HMG) or combination of both. Antagonists (Ganirelix) commenced when the leading follicle reached a diameter of 11 mm. Follicular growth was monitored via a transvaginal ultrasound and FSH dosage was adjusted accordingly. Trigger of ovulation with recombinant human chorionic gonadotropin (Ovidrel) or gonadotropin releasing hormone (GnRH) agonist (Triptorelin) was used for patients who were at risk of ovarian hyperstimulation syndrome (OHSS) when at least 3 follicles of 17 mm in diameter were achieved. Oocyte retrieval was performed 36th hour after trigger. Conventional IVF/ICSI was used for fertilisation.

Data and statistical analysis

The primary outcome was follicular output rate (FORT) which defined as the number of pre-ovulatory follicles (16-20mm) in response to follicle-stimulating hormone (FSH) administration, divided by the pre-existing antral follicle count (AFC) which presented in percentage.[13-14] The secondary outcomes were number of MII oocytes retrieved during oocyte pick-up. MII oocyte is defined as an oocyte at metaphase of meiosis II, exhibiting the first polar body and with the ability to become fertilized.[15-16] Additionally, number of good quality embryos on day 3 after fertilisation. Embryos were divided into those with good quality (embryos with at least 6 cells and a fragmentation rate <20%) and those with poor quality (<6 cell and a fragmentation rate > or equal to 20%)[2] rated by two experienced senior embryologists. Finally, we measured the endometrial thickness before embryo transfer with transvaginal ultrasound and our aim was more than 8mm. As patient and treatment specific parameters, we included age, body mass index, parity, smoking, alcohol consumption, causes of infertility which required IVF such male factor (based on sixth edition of the WHO laboratory manual for the examination and processing of human semen, 2021),[17] polycystic ovarian syndrome -PCOS (by Rotterdam criteria),[18-19] tubal factor infertility, endometriosis and unexplained infertility. The analysis also included duration of infertility in years, the duration of multinutrient or folic acid treatment (in days) until ovarian hyperstimulation, cumulative dose of FSH (in IU) and finally the total days of ovarian hyperstimulation.

Statistical analysis was performed using SPSS version 20. Variables were presented as mean ± standard deviation (SD) for numerical parameters, numbers and frequencies (%) for categorical parameters. For numeric variables, statistical analysis was performed using the Welch test for normally distributed and the Mann–Whitney U test in the case that there was no normal distribution. The chi-square or the Fisher's exact test was used for categorical variables. For all the statistical tests, the level of $p< 0.05$ was taken as significant.

Table I: Basic demographic characteristics of the IVF/ICSI study group and the control group

	Study group (n=25)	Control group (n=25)	p-value
Age, years	36.44 ± 3.33	35.32 ± 3.47	0.250[1]
Body mass index, kg/m^2	25.28 ± 4.12	24.80 ±4.36	0.691[1]
Parity	0.12 ± 0.33	0.48 ± 1.26	0.179[1]
Smoked (%)	0	0	NA
Alcohol consumption (%)	1 ± 4.0	0	>0.999[2]
Male factor (%)	14 (56.0)	11 (44.0)	0.396[3]
Polycystic ovary syndrome (%)	7 (28.0)	4 (16.0)	0.306[3]
Tubal factor (%)	2 (8.0)	7 (28.0)	0.138[4]
Endometriosis (%)	8 (32.0)	6 (24.0)	0.529[3]
Unexplained infertility (%)	3 (12.0)	5 (20.0)	0.702[4]

Data are presented as mean ± standard deviation for numerical parameters or numbers and frequencies (%) for categorical parameters; significance were tested using either.
[1] The Welch test, [2] The Fisher's exact test, [3] Chi-Square test, [4] Fisher's exact test

Table II: Infertility duration, treatment cycle and stimulation progress

	Study group (n=23)	Control group (n=22)	p-value
Duration of infertility in years, mean (±SD)	8.32 ± 3.50	6.08 ± 3.69	0.032[1]
Duration of treatment multinutrient vs Folic acid until ovarian hyperstimulation, days, mean (±SD)	52.96 ± 18.11	57.20 ± 24.89	0.495[1]
IVF treatment cycle, n (%)			
Cycle 1	13 (65.0)	18 (81.8)	
Cycle 2	5 (25.0)	3 (13.6)	0.486[2]
Cycle 3	2 (10.0)	1 (4.5)	
IVF treatment cycle, mean (±SD)	1.45 ± 0.69	1.23 ± 0.53	0.250[1]
Total dose of FSH/LH, mean (±SD)	2410 ± 656.82	2706.82 ± 536.15	0.119[1]
Days of stimulation, mean (±SD)	8.90 ± 2.13	9.68 ± 1.29	0.164[1]

Data are presented as mean ± standard deviation for numerical parameters or numbers and frequencies (%) for categorical parameters; significance were tested using either.
[1] The Welch test, [2] The Fisher's exact test

Table III: Major results of the IVF/ICSI study

	Study group (n=20)	Control group (n=22)	p-value
Follicular output rate, mean (±SD)	68.12 ± 19.47	64.91 ± 20.06	0.493[1]
Number of MII oocytes from mature follicles retrieved, mean (±SD)	6.65 ± 3.84	6.09 ± 3.01	0.626[1]
Number of good quality embryo on day 3 after fertilisation, mean (±SD)	4.00 ± 3.10	3.45 ± 2.30	0.549[1]
Endometrial thickness before embryo transfer, mean (±SD)	10.35 ± 1.32	10.36 ± 2.04	0.320[1]

Data are presented as mean ± standard deviation; significance were tested using either.
[1] The Welch test

RESULTS

Patient characteristics parameters were similar between the study and the control groups (Table I). All the patients in both groups underwent antagonist protocol. There was total 8 patients (study group n=5; control group n=3) were excluded from primary and secondary outcomes analysis (2 patients in study group had spontaneous pregnancy prior starting IVF and 3 patients converted to IUI during the course of stimulation, in control group 1 had spontaneous pregnancy and 2 patients refused IVF after completed treatment). According to patients' statements all women had correctly adhered to the supplementation regimens.

There is no difference in the mean age of women in both groups (36.44 ± 3.33years; 35.32 ± 3.47 years). The mean body mass index was comparable between the two groups. The duration of multinutrient and folic acid treatment in both groups, treatment cycle and stimulation progress were similar (Table II). There was no difference in the FORT after multinutrient supplementation as compared to the folic acid group. However, in this study we observed that the mean FORT in the multinutrient group was slightly higher compared to the control group (68.12 ± 19.47 vs 64.91 ± 20.06, p=0.493) (Table III). The mean number of MII oocytes retrieved from mature follicles and number of good quality embryo on day 3 after fertilisation were not statistically significant between the two groups (6.65 ±3.84 vs 6.09 ± 3.01, p=0.626 and 4.00 ± 3.10 vs 3.45 ± 2.30, p=0.549, respectively). In addition, there were no differences in endometrial thickness before embryo transfer in both groups (10.35 ± 1.32mm vs 10.36 ± 2.04mm, p=0.320). However, we perceived that the total dose of FSH and duration of

controlled ovarian stimulation were lower in the study group compared to control group (2410 ± 656.82 IU vs 2706.82 ± 536.15 IU, p= 0.119 and 8.90 ± 2.13 days vs 9.68 ± 1.29 days, p=0.164, respectively) (Table II).

DISCUSSION

This prospective controlled trial demonstrated that the use of multinutrient supplementation containing Omega 3, coenzyme Q10, folic acid, selenium, vitamin E and catechins led to higher follicular output rate compared to folic acid although did not reach statistical significance. Commonly, in assisted reproductive techniques, follicular recruitment and development in response to controlled ovarian hyperstimulation (COH) with gonadotropins are key elements and essential in the treatment of infertility.[20] In addition, ovarian responsiveness is one of the most commonly studied parameters in clinical research concerning IVF treatment, in which in our study we observed the FORT. Hypo-responsiveness to follicle stimulating hormone in controlled ovarian stimulation is a phenomenon manifests as a low follicles output rate (FORT) with a discrepancy between the relatively low number of pre-ovulatory follicles which develop following ovarian stimulation as compared to the number of antral follicles available at the start of stimulation. Normal responder is a phenomenon where the age of the women or ovarian reserve or previous ovarian response, predicted to result in a not too low, or too high ovarian response. The definition of normal responders is based on predicted response only; some women might have had an unexpected, exaggerated response while some others an unpredicted poor response. This limitation has been accepted, in absence of any better marker to denote 'normal responder'. On the other hand, hyper-responder is a condition where women were predicted to yield high ovarian response based on high AMH and/or high AFC and/or exaggerated follicular response in the previous cycle, except where a diagnosis of typical polycystic ovary syndrome (PCOS) was made. Multinutrient supplementation in women with fertility problems can help normalise trace element levels, which may have a positive impact on the quality of the microfollicular environment, and thus on oocyte and embryo quality, implantation, and live birth.[5,21] Camilia Bessow et al, mentioned that the number of oocytes retrieved is the main outcome measure of ovarian responsiveness to gonadotropin stimulation.[22] However, the number of pre-ovulatory follicles obtained at the end of COH is not a reliable reflection of the antral follicle sensitivity to FSH, and it is strongly correlated with the number of antral follicles before ovarian stimulation. Although maybe not only the number of antral follicles, but also their size, is important.[23] Therefore, we assessed the FORT and it is known to be correlated to the outcomes of IVF, including pregnancy rates. In our centre, not all patients had AMH before ovarian stimulation as it is indicated only for older women or for those who had prior ovarian surgery, as AMH can be one of the predictors of ovarian response to FSH. In contrast, AFC is routinely measured at the beginning of the cycle as there will be a cycle-to-cycle variation. However, we consider this as one of our study limitations and more future research is warranted on this matter.

As mentioned earlier, the mean number of MII oocytes retrieved from mature follicles in this study was not statistically significant between the two groups. Previous studies had shown that women with a diet high in EPA and DHA have the fewer MII oocytes.[24] Similarly, Hammiche et al. demonstrated that high intakes of EPA and DHA reduced oestrogen response and the number of follicles after ovarian stimulation.[25] Correspondingly, a study on rats fed a diet high in EPA and DHA showed a decline in frequency of ovulation.[26-27] The reduction in PGF2α involved in follicle growth and ovulation which was attributed to EPA and DHA may relatively explain the reduced number of MII oocytes.[28-29] Despite the inverse relationship between EPA intake and the number of MII oocytes from previous studies, it has been revealed that consuming monounsaturated fatty acids (MUFA) such as EPA could increase the fertilisation rate. Indeed, it was demonstrated that the gene expression of insulin-like growth factor-I (IGF-I) in granulosa cells increased by EPA could improve the fertilisation rate and embryo development.[30] More future research is needed to evaluate the impact of EPA and DHA on fertility outcome. We could not draw any conclusion from our study in regards to the use of Omega 3 fatty acid especially EPA and DHA as our sample size was small and we believe it's our study limitations.

Moreover, the use of multinutrient supplementation Fortelle + Omega -3 in this study revealed a slightly higher number of good quality embryos on day 3 after conventional IVF or ICSI treatment compared to the use of 5mg folic acid alone although not clinically significant. Kazem et al. in a prospective study demonstrated that multinutrient ingestion can significantly improve embryo quality in older women (>35 years) undergoing IVF/ICSI.[2] In addition, the use of the multinutrient resulted in a significantly higher fertilisation rate compared with folic acid only (66.7% vs 42.9% respectively) and a higher proportion of women with at least 1 good-quality embryo (58% vs 36%, respectively), supporting a beneficial effect of multinutrient supplementation on female fertility that may be due to the effects of the antioxidants within the formulation.[2]

Furthermore, we concluded that there is no significance achieved on endometrial thickness before embryo transfer between both groups. However, in a prospective randomised controlled trial by Cicek et al. demonstrated that in women with unexplained infertility undergoing controlled ovarian stimulation, supplementation with multinutrient especially vitamin E significantly increased endometrial thickness compared with no supplementation.[31] Supplementation was thought to improve the endometrial response via the antioxidant effects of vitamin E which has been shown to reduce oxidative stress and its anticoagulant effects by increasing endometrial and follicular blood supply.[31] A recent study by Marcus et al. revealed that the mean serum AMH levels and endometrial thickness values were significantly higher after 3 months micronutrient supplementation as compared to baseline.[4]

Coenzyme Q10 (CoQ10) is a lipid-soluble quinone, acting as an effective antioxidant, which prevents lipid peroxidation and DNA oxidation.[32] El Refaeey et al. in his study has

mentioned that oral CoQ10 supplementation in PCOS women increased the mean number of mature follicles (> 18 mm) and the ovulation rate per cycle (65.9% vs. 15.5%, p < 0.001) compared with no-treatment.[33] In another study in poor ovarian response women, CoQ10 increased the median number of oocytes retrieved and fertilised and increased the median number of day 3 high-quality embryos when compared with no-treatment.[34-35]

Selenium (Se) is an important micronutrient for several vital functions, such as deoxyribonucleic acid (DNA) synthesis, modulation of the thyroid metabolism and an antioxidant.[36] Luiz G et al. in his systematic review revealed that selenium supplementation increases number of oocytes and follicles after stimulation in IVF, increases concentration of antioxidants in follicular fluid, maintains serum selenium level during pregnancy and decreases antithyroid antibody levels in women with autoimmune thyroid disease.[37]

Vitamin E's antioxidant properties may protect both the mother and baby throughout pregnancy by acting as a chain-breaking antioxidant and the body's primary lipid peroxyl radical scavenger, hence reducing the likelihood of complications during pregnancy.[38] Cicek et al. suggested that vitamin E, through its antioxidant action, may enhance the endometrial environment and thickness in women with unexplained or idiopathic infertility.[31] Bahadori et al. had mentioned that optimal concentration of Vitamin E in follicular fluid essential for the highest percentage of metaphase II oocytes and higher quality embryos.[39]

Green tea catechins have been reported to exert antioxidative effects in humans.[2] In animal study with endometriosis, it enhances anti proliferative properties by reducing development of endometriosis lesions and anti-angiogenic properties by reducing angiogenesis of endometriotic lesions. On the other hand, in a clinical trial patient with polycystic ovarian syndrome (PCOS) green tea catechins decreases rises of testosterone and reduces fasting insulin on overweight and obese PCOS women.[40]

It is well established that folic acid supplementation is vital to reduce the risk of neural tube defects. Patients in the control group were given Folic Acid 5mg for at least 1 month prior to ovarian stimulation. We choose to use Folic acid 5mg as it is the only preparation widely available in our hospital as well as the Ministry of Health Malaysia.

Finally, Omega 3 fatty acids introduced through a multivitamin or single compound supplementation may be beneficial in terms of fertilisation rates and embryo quality as well as a high intake of oils rich in omega-3 was observed to improve oestradiol levels, ovulation, follicles development and embryo morphology.[2,26]

In this pilot study, we focused on follicular output rate as the major outcome parameter. Although multinutrient widely researched in female fertility outcome, no studies reported on the FORT, an important marker for IVF outcomes. This study was aimed at evaluating the FORT between the both study groups. In this study we observed that the mean FORT in the multinutrient group was slightly higher compared to the control group although no statistical significance. Future studies are therefore warranted to confirm the clinical effect of the tested multinutrient supplementation mainly in FORT.

Our results must be interpreted with care due to the design as a pilot study, although controlled no proper randomization done with a small sample size without a prior sample size calculation which we consider to be the limitations of the study. Recruited women in both groups might have different dietary profiles with various nutritional status, which such bias would likely lead to a reduction of the beneficial effects observed in the study group. Ideally, to test the serum levels of the supplemented multinutrient before and after treatment which was not done in our trial. Notably, we assume that some women of the control group who had informed about the beneficial effect of multinutrient might have bought the preparation or another multinutrient similar to that on their own. Such a bias would likely lead to a reduction of the beneficial effects observed in the study group. Last but not least, the embryologist's intraobserver variability in the scoring was not evaluated, which might have introduced some bias as well. This study provides preliminary evidence that multinutrient supplementation increases follicular output rate, higher number of MII oocytes and good quality embryos on day 3 after fertilisation though no clinical significance. We perceived that the total dose of follicle stimulating hormone and duration of controlled ovarian stimulation were lower in the study group. There is a need for a multicenter, randomised controlled trial with larger sample size to validate the positive effects of multinutrient in female fertility.

CONCLUSION

In conclusion, a multinutrient supplementation such as Omega 3, coenzyme Q10, folic acid, selenium, vitamin E and catechins given for a minimum of 28 days, may have a positive effect on FORT. More research is warranted to prove this effect.

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DISCLOSURE STATEMENT

The authors report no conflicts of interest.

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