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Association of Micronutrient Intakes With Female Infertility: Review of Recent Evidence

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Context: Oxidative stress is one of the factors related to the pathogenesis of fertility disorders such as idiopathic infertility, polycystic ovarian syndrome, and endometriosis. Hence, the role of micronutrients has attracted the attention of researchers to the extent that some studies have investigated the role of vitamins and minerals in the risk of female infertility. This study aimed to summarize the literatures regarding the association between micronutrient intakes and female infertility.

Evidence Acquisition: Literature searching for studies on female infertility and micronutrient intakes, published between January 1984 and November 2014, was performed using the PubMed and Embase databases. The bibliographies of included studies were also searched for additional references.

Results: About 100 articles were identified and after the elimination of irrelevant studies, 28 related studies available for review were examined. Researches have demonstrated that in women with idiopathic infertility, total antioxidant status and levels of malondialdehyde (MDA) and homocysteine (Hcys) were respectively lower and higher. Moreover, some studies have shown that higher intake of iron, folic acid, and vitamins D and E may play a beneficial role in female infertility. However, a number of other studies have not attained such results for vitamin C and N-acetylcysteine.

Conclusions: Studies revealed that micronutrient intakes play a substantial role in preventing or facilitating female infertility. Further studies are needed to evaluate this association.

*Keywords:*Micronutrients; Oxidative Stress; Antioxidants; Vitamins; Minerals; Infertility, Female

1. Context

Infertility is defined as at least 12 months unsuccessful attempt to conceive for women younger than 35 years or at least 6 months for a women older than 35 years old (1). Generally, about 15% of couples have been dealt with this condition during their reproductive lifetime (2). Assisted-reproduction methods are considered as first-line treatment of infertility (3); however, their considerable costs and adverse events make them less accessible for many couples (3).

Although the association of dietary factors with human infertility remains unclear, some researches have reported improve female infertility by consuming some micronutrient (3). Some studies have shown that micronutrient supplements users have higher pregnancy rates even if they have fertility disorders (4). Moreover, about 10% to 15% of infertilities are considered idiopathic, which may be caused by oxidative stress (5). Additionally, it has been suggested that oxidative stress plays a role in the pathophysiology of unexplained infertility, endometriosis, polycystic ovarian syndrome (PCOS), and tubal and peritoneal factor infertility (5). Therefore, numerous studies have investigated intervention strategy of micronutrient supplementation in oxidative status of infertile women (6-8). On the other hand, there is some evidence of micronutrients role in ovarian hormones secretion and uterus structure (9). The review comprehensively explores the literature for evidence of the role of macronutrients such as group B vitamins, vitamins C, E, and D, selenium, iron, and magnesium in female infertility.

2. Evidence Acquisition

In order to examine the association between micronutrients and female infertility, articles with case-control, descriptive, cohort, and interventional (clinical trials) design, published between 1984 and November 2014, were accessed through PubMed and Embase databases, using keywords such as "oxidative stress", "antioxidant", "vitamins", "minerals", and "female infertility". Studies that had investigated association between micronutrients and female infertility are shown in Table 1, Supporting studies were excluded from the Table 1.

Table 1. Characteristics and Results of Studies on the Association Between Micronutrients and Female Infertility [a]

	Study		
	Design	**Comment**	**Results**
Paszkowski et al. (10)	Case-Control	112 women with idiopathic infertility, tubal infertility vs healthy women	Lower levels of selenium and glutathione peroxidase in patients with idiopathic infertility
Polak et al. (11)	Case-Control	53 women with fertility disorders vs 13 healthy women	Lower total antioxidant status in idiopathic infertile patients
Madhur Mahesh Gupta (12)	Case-Control	50 women with idiopathic infertility vs 50 healthy women	Higher levels of serum MDA and Hcys in infertile patients
Howard et al. (6)	Clinical trial	Supplementation with magnesium and selenium in infertile women	100% of infertile patients become pregnant within the 8 months
Badawy et al. (8)	Clinical trial	NAC supplements in combination with clomiphene citrate in infertile women	No significant differences were observed with NAC supplements
Mier-Cabrera et al. (7)	Clinical trial	Supplementation with vitamins C and E in infertile women	No significant differences were observed in pregnancy rates
Cicek et al. (9)	Clinical trial	Vitamin E supplements in combination with clomiphene citrate in infertile women	Increased endometrial thickness in vitamin group
Altmae et al. (13)	Case-Control	Folate-metabolizing gene variations and unexplained infertility	Significant differences in the frequencies of heterozygous genotype between controls and infertile patients
Thaler et al. (14)	Clinical trial	The effects of 677 C>T mutations of *MTHFR* gene on ovarian responsive to r-FSH	Less r-FSH was required in patients with 677 c homozygote
Szymanski et al. (15)	Clinical trial	Folic acid supplement in IVF	Higher degree of maturity of oocytes in vitamin group
Chavarro et al. (3)	Cohort	Supplementation with multivitamin and ovulatory infertility risk	RR=0.59 for women using 6 or more tablets weekly
Chavarro et al. (16)	Cohort	the association of iron intake with ovulatory infertility	RR=0.53 for women in highest quintile of total iron intake
Li et al. (32)	Cross-Sectional	The prevalence of vitamin D deficiency in 1192 infertile patients	68.6% and 22.2% of patients had been considered insufficient and deficient, respectively
Ozkan et al. (18)	Cohort	Association between serum levels of vitamin D and IVF outcomes	Each ng/ml increase in 25(OH)-D levels in follicular fluid had correlated with 6% increase in clinical pregnancy rates

[a] Abbreviations: NAC, N-acetylcysteine; IVF, in vitro fertilization; MDA, malondialdehyde; Hcys, homocysteine.

3. Results

3.1. Oxidative Stress and Female Infertility

In the human body, reactive oxygen species (ROS) are formed under physiologic and pathologic condition (5, 19). They can be produced from endogenous sources, for instance during aerobic metabolism and due to different metabolic pathways, or as part of defense mechanism of the body (5). In addition, ROS can be formed exogenously as a result of numerous environmental pollutants and by cigarette and alcohol use (5). Data have been implicated that regulated levels of ROS in ovaries, endometrium, fallopian tube, embryos, and peritoneal fluid play a role in tissue remodeling, hormone signaling, ovarian steroidogenesis, folliculogenesis, maturation of oocyte, tubal function, and cyclical and endometrial changes (5, 20). However, pathologic levels of ROS have resulted in considerable damage to cell structure (11). Moreover, some evidence reported that ROS are involved in developing female infertility (19-21). Substantial increase in OS levels has been linked with damage to the DNA of the oocytes and spermatozoa, resulting in defective fertilization (20). Even if fertilization is achieved, ROS induce apoptosis leading to implantation failure, abortion, and embryo fragmentation (21). Under normal condition, antioxidants are capable to inhibit ROS production, scavenge presenting free radicals, and trigger the repair of cell structure damage, which are induced by ROS (21). There are two nonenzymatic and enzymatic antioxidants (5).

The first group includes vitamin C, vitamin E, selenium, zinc, beta-carotene, carotene, taurine, hypotaurine, systeamine, and glutathione (5). The second group consists of super oxide dismutase (SOD), catalase, glutathione peroxidase, glutaredoxin, and glutathione reductase (5). Total antioxidant capacity (TAC) is often considered as the degree of antioxidant defense (11). A study by Paszkowski et al. showed that selenium and glutathione peroxidase levels of peritoneal fluid in cases with idiopathic infertility were considerably lower than were in those with tubal infertility and control group (10). Another case-control study on 53 women with idiopathic infertility, tubal infertility, and mild endometriosis and 13 healthy controls revealed that those with unexplained infertility had a significantly lower total antioxidant status (049 ± 0.21 mmol/L) compared with both fertile women (0.67 ± 0.24 mmol/L; P = 0.02) and women with tubal infertility (0.76 ± 0.26 mmol/L; P = 0.001) (11). The antioxidant status of peritoneal fluid did not differ significantly between women with endometriosis (0.61 ± 0.2 mmol/L), those with tubal infertility, and control group (P > 0.05). A study on 50 women with unexplained infertility and 50 control women suggested that serum MDA and Hcys levels in the cases (2.29 ± 0.45 nmol/L and 13.24 ± 5.27 μmol/L, respectively) were significantly higher than those in control group were (1.76 ± 0.20 nmol/L and 6.65 ± 0.89 μmol/L, respectively; P < 0.001) (12). In a study on 12 idiopathic infertile women who had low red blood cells' magnesium (RBC-Mg) levels, a daily intake of 600 mg vitamin C and 200 μg selenium as selenomethionine in a period of two months resulted in normalizing RBC-Mg levels. All 12 infertile women became pregnant within eight months of normalizing their RBC-Mg levels and have produced healthy babies (6).

A randomized trial investigated 404 and 400 women with unexplained infertility as study and control groups, respectively. The study group was treated with clomiphene citrate (50-mg tablets) twice daily and with N-acetylcysteine (1200 mg, daily) for five days, starting on second day of the cycle, and the control group was treated with clomiphene citrate and sugar powder as placebo. No significant differences were observed between two groups in the number and size of follicles, mean estrogen levels, serum progesterone, and endometrial thickness (P > 0.05). In addition, the authors reported that pregnancy rates were comparable in both group (27% vs. 22.2%) (8). In another randomized trial, 34 women with endometriosis, receiving daily 343 mg of vitamin C and 84 mg of vitamin E or placebo, were studied in a period of six month (7). Plasma and peritoneal fluid of MDA and lipid hydroperoxide (LOOH) were assessed in all women before and after the intervention. After four month, the study group had lower levels of MDA and LOOH (16.89 ± 4.54 μmol/L and 8.45 ± 1.39 μmol/L, respectively.) compared to the control group (25.8 ± 7.22 μmol/L and 10.77 ± 1.82 μmol/L, respectively). The authors reported that there was a statistically significant difference in plasma concentration of LOOHs and MDA between study groups at four and six month (P < 0.05). However, when pregnancy rates taken into account, no significant differences were observed between two groups (19% vs. 12%). Another randomized trial was conducted on 53 women as the vitamin group and 50 women as placebo group, all with unexplained infertility (9). The vitamin group underwent ovulation induction with clomiphene citrate with a daily intake of 400 IU vitamin E, whereas the control group underwent controlled ovarian stimulation without vitamin E. Vitamin E administration had started from the third to fifth days of the menstrual cycle until the injection day of human chorionic gonadotropin (hCG) of the controlled ovarian stimulation. Receiving vitamin E was significantly correlated with endometrial thickness on the day of hCG administration (9.6 ± 2.1 and 8.2 ± 2 in vitamin E and placebo groups, respectively; P = 0.001), while, it had no association with the implantation and the ongoing pregnancy rates (P < 0.05). Moreover, researchers have shown that thin endometrium is associated with unexplained infertility (9). Hence, consuming vitamin E may enhance the endometrial response in this condition through antioxidants and anticoagulant effects.

To conclude, the findings of different studies revealed that low antioxidant status might be involved in fertility disorders. Moreover, some antioxidant supplementation may improve this condition. Nevertheless, more randomized controlled trial is needed to investigate the efficacy of supplementation with antioxidants in female fertility disorders.

3.2. Vitamin B Group Intake and Female Infertility

Folic acid and folate play a role in the remethylation of Hcys to methionine, which is catalyzed by methionine synthase and 5,10-methylentetrahydrofolate reductase (MTHFR); moreover, they are the precursors for the coenzyme tetrahydrofolate in transferring of single-carbon in the metabolism of amino acids and nucleic acids (22). An insufficient dietary intake of folate leads to decrease in DNA biosynthesis and consequently cell division, resulting in anemia, leucopenia, thrombocytopenia, and other adverse effects (22). Moreover, many studies have reported that folic acid supplementation is involved in a reduction of neural tube defects (NTD) incidence (22, 23). Another study investigated the association between folate-metabolizing gene variations and unexplained infertility (13). Blood samples were collected from 71 women with idiopathic infertility to assess the polymorphism genotyping. Data from target patients were compared with data from cross-sectional population studies performed in the same region. Considerable differences in the frequencies of heterozygous genotype between controls and infertile patients were observed regarding to polymorphism *MTHFR* 677 C/T (43.6% vs 32.4%, respectively; P = 0.043) and *MTHFR* 1793 G/A (9.1% vs 1.4%, respectively; P = 0.012). Another study investigated the effects of

677 C > T mutations of *MTHFR* gene on ovarian responsive to recombinant follicle-stimulating hormone (r-FSH) in 105 infertile patients (14). Data had shown that less r-FSH was required in patients with 677 c homozygote ($P < 0.02$). Those women also produced considerably more oocytes ($P < 0.04$) and higher maximal serum estradiol concentration ($P < 0.002$). Data also indicated that mutation of the *MTHFR* 677 C > T may influence ovarian responsiveness.

Some studies have investigated the effects of supplementation with folic acid in women undergoing in vitro fertilization (IVF) (15). A randomized trial evaluated the efficacy of folic acid supplement in reducing the Hcys levels in follicular fluid of 40 patients who were qualified for IVF-Embryo Transfer (ET) (15). During the trial, women on the supplement arm (n = 20) had oocytes with better quality and higher degree of maturity as well as lower levels of Hcys in both follicular fluid and serum compared to women in the placebo arm ($P < 0.05$). In a prospective cohort study on 18555 healthy married women, an inverse association was observed between frequency of multivitamin consumption and ovulatory infertility during eight years of follow-up (3). The multivariate relative risk (RR) was 0.59 for six or more tablets, 0.69 for three to five tablets weekly, and 0.88 for less than two tablets per week consumption in comparison to women who did not consume those supplement ($P < 0.001$). Data suggested that folic acid was involved in the association supplementation with multivitamin and ovulatory infertility risk. However, such an association was not found for vitamin B5. Moreover, it has been reported that after adjustment for age and energy, intakes of vitamins B1, B2, B6, B12, folic acid, and niacin had inverse association with risk of ovulatory infertility, whereas pantothenic acid intake had no effects on this kind of infertility. Surprisingly, after accounting for known and suspected infertility risk factors, especially iron intake, only intake of folic acid was associated with a reduction in ovulatory infertility risk. The authors reported that supplementation with multivitamin (at least three times a week) would lead to reduced risk of ovulatory infertility by 20%. Because there is growing literature on the role of folic acid, in a reduction the risk of NTD and also other congenital malformation either by itself or as part of multivitamins (24, 25), women who attempting to become pregnant should consider consuming a multivitamins as they might enhance fertility.

3.3. Iron Intake and Female Infertility

Numerous studies have shown that iron may be involved in ovulatory function and fertility (16, 26). Studies have reported that the most prevalent nutritional deficiency worldwide is iron deficiency (16). One of the high-risk groups for this condition is women of reproductive age (16). Moreover, supplementation with iron have been correlated with diminish in prevalence of iron deficiency (16). The possible role of iron status in female reproduction is addressed by researches on the presence of iron-transporting proteins in ovarian cells (26, 27). Data demonstrated that both transferrin and its receptor are found in granulose cells and oocytes (27). Similarly, some evidence has shown that granulose cells are capable to synthesize transferrin, which could be translocated to the oocyte (26). However, some data suggested that ovarian transferrin and transferrin receptors might not participate in local iron metabolism (26). It has been shown that these proteins are needed for ovum development and required to supply the increased iron demand in the developing follicle (27). A cohort study on 18555 healthy married women investigated the association of iron intake with ovulatory infertility (16). The study reported that after being multivariable adjusted, the RR was 0.53 for women in highest quintile of total iron intake. The RRs for highest quintile of heme and non-heme iron intake were 1.31 and 0.6, respectively. Therefore, an inverse association was observed between ovulatory infertility and both total iron intake and non-heme iron intake. Although heme iron intake was accompanied with greater risk of ovulatory infertility. As far as iron supplement is concerned, an inverse dose-dependent association was reported. Multivariable adjusted RR for high and low content supplement users were 0.38 and 1.13, respectively, compared to non-users (referent group). Furthermore, finding from several studies indicate that women with celiac disease, which is usually linked with iron stores depletion and also other micronutrients deficiencies, have impaired reproductive function, e.g. delayed menarche, early menopause, and idiopathic infertility (28, 29). In addition, it has been reported that some of infertile women with celiac disease had signs of iron deficiency such as iron deficiency anemia and low ferritin levels, while no other nutrient deficiencies were observed (29).

Taken as a whole, supplementation with iron should be considered by women planning to become pregnant because it might prevent iron deficiency and facilitate fertility in a dose-dependent manner.

3.4. Vitamin D Status and Female Infertility

Low levels of vitamin D have been linked to pregnancy complications, congenital rickets and fractures in the newborn and poor outcomes in assisted reproduction (30). Generally, experts believe that serum levels < 20 ng/mL are deficient and serum levels < 32 ng/mL have been considered inadequate. A cross-sectional study evaluates the prevalence of vitamin D deficiency in patients who underwent fertility treatment at a large private practice in Northern California. Data had indicated that the median vitamin D levels of 1192 patients were 27 ng/mL; moreover, 68.6% and 22.2% of patients were considered insufficient and deficient, respectively. Furthermore, some evidence has slinked lower levels of vitamin D to PCOS phenotype (30). Several studies have been demonstrated that vitamin D levels in patients with PCOS were

less than that in controls (30, 31). In addition, deficiency of vitamin D may be associated with insulin resistance, obesity, and metabolic syndrome, all of which can result in ovulatory dysfunction (31). A prospective cohort study investigated the association between serum levels of VD and IVF outcomes in 84 infertile women presenting for IVF (18). The authors reported that those who had higher vitamin D levels in their serum and follicular fluid were considerably more likely to achieve clinical pregnancy following IVF-ET. Interestingly, these data suggested that each ng/mL increase in 25(OH)-D levels in follicular fluid corresponded to 6% increase in clinical pregnancy rates.

In conclusion, vitamin D assessment should be taken to account as part of infertility treatment as vitamin D supplement in vitamin D-depleted cases may lead to enhanced fertility outcome and overall health.

4. Conclusions

The evidence from studies suggests that an imbalance in antioxidant system, mainly in peritoneal fluid, may be associated with idiopathic female infertility (10, 11). Similarly, the oxidative stress markers such as MDA, LOOH, and Hcys were higher in infertile women (12, 19). Moreover, some studies revealed that *MTHFR* 677 C/T polymorphism may affect ovarian responsiveness to r-FSH through its effects on serum folate levels (14). Some data indicated that magnesium and selenium supplementation resulted in increased levels of RBC-Mg and serum glutathione peroxidase and hence, improved fertility rates (6) while other researchers revealed that supplementations with vitamin C and N-acetylcysteine were unrelated to improvements in fertility (7, 8). On the other hands, some data suggested that vitamin E supplement was associated with increase endometrial thickness (9). In addition, data from cohort studies indicated that total iron intake and intake of non-heme iron as well as multivitamin consumption were associated with lower risk of ovulatory infertility (3, 16). Furthermore, sufficient levels of vitamin D as well as folic acid supplement had resulted in increased clinical pregnancy rate (15, 18).

Authors' Contributions

Banafshe Hosseini and Ghazaleh Eslamian conceptualized the study, searched online databases, wrote the manuscript, and read and approved final manuscript.

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