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# Vitamin and Carotenoid Intake and Outcomes of In Vitro Fertilization in Women Referring to an Italian Fertility Service: A Cross-Sectional Analysis of a Prospective Cohort Study

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**Abstract:** Background: Nutrition may impact reproductive health and fertility potential. The role of dietary antioxidants in affecting conception and birth outcomes is a topic of emerging interest. Methods: This cross-sectional analysis from a prospective cohort study aims to explore the relationship between the intake of antioxidants, vitamins, and carotenoids and the outcomes of assisted reproduction techniques. Information on the socio-demographic characteristics, health histories, lifestyle habits, and diet information of subfertile couples referred to a fertility center was obtained. Results: A total of 494 women were enrolled. According to the four IVF outcomes considered, 95% of women achieved good quality oocytes, 87% achieved embryo transfer, 32.0% achieved clinical pregnancies, and 24.5% achieved pregnancy at term. Associations were found between age and the number of good quality oocytes ($p$ = 0.02). A moderate level of physical activity in the prior 5 years was associated with a better rate of achieving clinical pregnancy ($p$ = 0.03). Smoking habits, alcohol intake, and caffeine consumption did not show associations with any outcome. No associations were found, even after accounting for potential confounders, with the intake of vitamins C, D, E, and α-carotene, β-carotene, beta-cryptoxanthin, lutein, and folate. Conclusion: Further research is needed to understand how antioxidant intake may have a role in modulating fertility.

**Keywords:** nutrition; antioxidants; assisted reproductive techniques; infertility

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## 1. Introduction

Nutritional factors may contribute in significant ways to determining reproductive health and thus influencing fertility in humans [1]. The role of nutrients in processes such as implantation, placental growth, and angiogenesis is well-recognized, as is the transfer of nutrients from the mother to the fetus. Up to 15% of couples experience infertility worldwide, particularly in developed countries [2]. Unbalanced nutrition is defined as excessive energy intake as well as inadequate micronutrient and macronutrient intake. Malnutrition, together with the reduction in physical activity, contributes to body fat increase and to the development of non-communicable diseases [3]. Lifestyle factors, such as smoking, alcohol abuse, and poor nutritional intake, can also negatively impact fertility potential [4].

The role of dietary antioxidant intake in affecting conception and birth outcome is a topic of emerging interest [5]. Oxidative stress (OS) and low antioxidant status may be associated with infertility of both known and idiopathic origins [5]. Growing evidence indicates that OS impairs gamete and embryo development outcomes during assisted reproductive procedures techniques (ART) [6]. A lower total antioxidant status has been reported in infertile women [7]. Thus, it has been suggested that supplementation with antioxidants may improve infertility treatment outcomes [8].

Vitamin C, α- and β-carotene, vitamin E, and β-cryptoxanthin have antioxidant activities and may thus provide cellular defense against reactive oxygen species (ROS) that damage DNA [9]. Folate is important for the synthesis of DNA, transfer RNA, and the amino acids cysteine and methionine, and folate is a relevant contributor of reproduction. Folic acid, in the form of folate, effectively scavenges oxidizing free radicals, and, as such, it too can be regarded as an antioxidant [10].

Various randomized controlled trials (RCTs) have investigated the role of antioxidant supplementation and of a good antioxidant's status, but findings are still inconsistent. For instance, Ruder et al. showed that vitamin C, β-carotene, and vitamin E were related to better ART outcomes in subgroups of women defined by BMI or age [11]. At the same time, another group of authors found that vitamin C supplementation increased pregnancy rates primarily among non-smokers [12]. Notably, the high intake of some vitamins (e.g., vitamin A) may result in adverse reproductive outcomes. This has led to the hypothesis that relation between micronutrients and ART outcomes may not be linear [13]. In vitro and animal studies have focused on several possible ways through which OS may affect fertility and early pregnancy loss. However, no study has directly addressed the effects of OS on fertility in women, although a recent review has addressed the effects of folate, zinc, and antioxidants in the pathogenesis of subfertility [14]. Studying diet in relation to fertility outcome raises interest, as it is a potentially modifiable predictor of a treatment outcome. The present study elaborates on the relationship between lifestyle and micronutrient intake with four main outcomes: (1) good quality oocytes, (2) successful embryo transfer, (3) clinical pregnancies, and (4) successful term pregnancies in women of subfertile couples presenting to an Italian Fertility Clinic and candidates for in vitro fertilization (IVF).

## 2. Materials and Methods

*Sample Size*

The number of 494 patients allows a power (1-β) of 0.77, 0.89, 0.86, and 0.66, for incidences of the reference group of 0.2, 0.4, 0.6, and 0.8, respectively, assuming a relative risk = 1.5 and α = 0.05. The calculations are the same as those in the comparing proportions for two independent samples setting: $p1 = p0$ and $p2 = p0 * RR/(1 + p0 * (RR - 1))$ [15].

Methods for this study are described elsewhere [16]. Briefly, both partners of couples who agreed to participate were interviewed by trained personnel. Information on general socio-demographic characteristics, personal and health histories, and habits (including smoking, physical activity, alcohol intake, and methylxanthine-containing beverage consumption) was collected.

Through a reproducible and validated food frequency questionnaire (FFQ), information on diet was obtained. The FFQ included 78 foods items or various food groups—such as the major sources of animal fats (i.e., red meat, milk, cheese, ham, and salami), folate, vitamins (vegetables and fruit), pasta and bread, cake, sweets, chocolate, and fish—and the most common Italian recipes [9–11]. Patients were asked to report their usual weekly food consumption in the previous year. The FFQ included the average weekly consumption of 78 food items or food groups. Energy levels and mineral intake, as well as macronutrient and micronutrient intake, were estimated using the most recent update of an Italian food consumption database [12].

Subjects were interviewed on their usual consumption of alcoholic beverages. Considering the different ethanol concentrations, one drink corresponded to approximately 125 mL of wine, 330 mL of beer, and 30 mL of hard liquor (i.e., about 12.5 g of ethanol). Total alcohol intake, expressed in grams of ethanol per day (g/day), was calculated as the sum of all reported alcoholic beverages. Patients who abstained from drinking for life were categorized as "never drinkers"; individuals who had abstained from drinking for at least 12 months at the time of the interview were categorized as "ex-drinkers." For this study, we considered the subjects in this category as "abstainers."

Information on coffee and other methylxanthine-containing beverages (tea, cocoa, and decaffeinated coffee), was also collected, and the average number of cups consumed per day was registered. Caffeine intake from coffee (60 mg per cup), cappuccino (75 mg per cup), tea (45 mg per cup), decaffeinated coffee (4 mg per cup), and chocolate (6 mg/10 g) was calculated [13]. Satisfactory reproducibility of questions on self-reported smoking and drinking habits in our study populations was previously reported [14]. Patients were managed according to a standardized clinical protocol [17]. A serum hCG assessment to detect pregnancy was performed 14 or 16 days after an ovulation triggering or luteinizing hormone (LH) surge. Women with positive human chorionic gonadotropin (hCG) values underwent a transvaginal sonography three weeks later. Clinical pregnancy was defined as the presence of at least one intrauterine gestational sac.

We report data on the female partners in relation to the first IVF outcome. The study was proposed during the diagnostic phase, and the interview was administered on the oocyte retrieval day to collect information on general socio-demographic characteristics, health histories, and lifestyle habits. Couples that did not speak Italian were excluded.

The participation rate was close to 95%. Body mass index (BMI) was classified through the World Health Organization (WHO) indications [18]. Occupational physical activity (PA) was categorized as heavy/very heavy, light/moderate, and mainly standing or mainly sitting. Leisure PA was defined in terms of hours/week: $<2$, between 2 and 4, and $\geq 5$. We noted the smoking habits collected as never, former, or current; we also noted the number of cigarettes smoked daily and the duration of smoking. Information on alcohol and caffeine intake was also collected, as previously reported. A previously validated FFQ [19–21] was used to obtain information on diet. Using information obtained from a validated database, we estimated the macronutrient, calorie, and micronutrient intakes, particularly of vitamins (C, D, E), α-carotene, β-carotene, beta-cryptoxanthin, lutein, and folate. The content of each micronutrient in the foods was calculated as follows: frequency of consumption of the food, multiplied by the portion of the food consumed given from the FFQ questionnaire, multiplied by the mean content of the micronutrient in the specific food. The FFQ explored the average weekly consumption of 78 food items or food groups and beverages per year. Intakes that were lower than once per week, but at least as high as once per month, were coded as 0.5 per week. To account for seasonal consumption, we considered weekly consumption of vegetables/fruits available in limited periods during the year and weighted for months of consumption. Daily energy and mineral, macronutrient, and micronutrient intakes were estimated using the most recent update of an Italian food composition database [22].

## 3. Results

In total, 494 women were included in the present study. The lifestyles, anthropometric characteristics, and clinical characteristics of the women are presented in Table 1 according to the considered IVF outcomes.

**Table 1.** Baseline characteristics by IVF outcomes.

| | **Good Quality Oocytes** | | | | | **Embryo Transfer** | | | | | **Clinical Pregnancy** | | | | | **Pregnancy at Term *** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **No N = 25** | | **Yes N = 469** | | | **No N = 65** | | **Yes N = 429** | | | **No N = 336** | | **Yes N = 158** | | | **No N = 372** | | **Yes N = 121** | | |
| | **N** | **%** | **N** | **%** | ***p*-Value **** | **N** | **%** | **N** | **%** | ***p*-Value **** | **N** | **%** | **N** | **%** | ***p*-Value **** | **N** | **%** | **N** | **%** | ***p*-Value **** |
| Age (years) | | | | | 0.0223 | | | | | 0.0540 | | | | | 0.0004 | | | | | <0.0001 |
| 1. <35 | 3 | 12.0 | 133 | 28.4 | | 10 | 15.4 | 126 | 29.4 | | 79 | 23.5 | 57 | 36.1 | | 87 | 23.4 | 49 | 40.5 | |
| 2. 35–39 | 11 | 44.0 | 234 | 49.9 | | 36 | 55.4 | 209 | 48.7 | | 165 | 49.1 | 80 | 50.6 | | 186 | 50.0 | 58 | 47.9 | |
| 3. ≥40 | 11 | 44.0 | 102 | 21.7 | | 19 | 29.2 | 94 | 21.9 | | 92 | 27.4 | 21 | 13.3 | | 99 | 26.6 | 14 | 11.6 | |
| Education | | | | | 0.6528 | | | | | 0.3585 | | | | | 0.7635 | | | | | 0.7672 |
| 1. College | 11 | 44.0 | 228 | 48.6 | | 28 | 43.1 | 211 | 49.2 | | 161 | 47.9 | 78 | 49.4 | | 181 | 48.7 | 57 | 47.1 | |
| 2. Degree | 14 | 56.0 | 241 | 51.4 | | 37 | 56.9 | 218 | 50.8 | | 175 | 52.1 | 80 | 50.6 | | 191 | 51.3 | 64 | 52.9 | |
| BMI | | | | | 0.6933 *** | | | | | 0.3883 | | | | | 0.9705 | | | | | 0.5923 |
| <18.5 | 1 | 4.0 | 41 | 8.7 | | 5 | 7.7 | 37 | 8.6 | | 29 | 8.6 | 13 | 8.2 | | 35 | 9.4 | 7 | 5.8 | |
| 18.5–24.9 | 17 | 68.0 | 335 | 71.4 | | 47 | 72.3 | 305 | 71.1 | | 241 | 71.7 | 111 | 70.3 | | 261 | 70.2 | 91 | 75.2 | |
| 25.0–29.9 | 5 | 20.0 | 63 | 13.4 | | 11 | 16.9 | 57 | 13.3 | | 45 | 13.4 | 23 | 14.6 | | 52 | 14.0 | 15 | 12.4 | |
| 30.0+ | 1 | 4.0 | 28 | 6.0 | | 1 | 1.5 | 28 | 6.5 | | 19 | 5.7 | 10 | 6.3 | | 22 | 5.9 | 7 | 5.8 | |
| Missing | 1 | 4.0 | 2 | 0.4 | | 1 | 1.5 | 2 | 0.5 | | 2 | 0.6 | 1 | 0.6 | | 2 | 0.5 | 1 | 0.8 | |
| Smoking habit | | | | | 0.7627 | | | | | 0.8722 | | | | | 0.6049 | | | | | 0.6024 |
| Never | 15 | 60.0 | 258 | 55.0 | | 34 | 52.3 | 239 | 55.7 | | 188 | 56.0 | 85 | 53.8 | | 210 | 56.5 | 63 | 52.1 | |
| Current | 5 | 20.0 | 86 | 18.3 | | 13 | 20.0 | 78 | 18.2 | | 64 | 19.0 | 27 | 17.1 | | 68 | 18.3 | 22 | 18.2 | |
| Former | 5 | 20.0 | 125 | 26.7 | | 18 | 27.7 | 112 | 26.1 | | 84 | 25.0 | 46 | 29.1 | | 94 | 25.3 | 36 | 29.8 | |
| Physical activity (5 years—work) | | | | | 0.9531 | | | | | 0.2753 | | | | | 0.9397 | | | | | 0.9643 |
| 1. (Very) heavy/moderate | 7 | 28.0 | 139 | 29.6 | | 22 | 33.8 | 124 | 28.9 | | 100 | 29.8 | 46 | 29.1 | | 111 | 29.8 | 34 | 28.1 | |
| 3. Mainly standing | 5 | 20.0 | 100 | 21.3 | | 9 | 13.8 | 96 | 22.4 | | 73 | 21.7 | 32 | 20.3 | | 79 | 21.2 | 26 | 21.5 | |
| 4. Mainly sitting | 13 | 52.0 | 228 | 48.6 | | 34 | 52.3 | 207 | 48.3 | | 163 | 48.5 | 78 | 49.4 | | 182 | 48.9 | 59 | 48.8 | |
| Missing | 0 | - | 2 | 0.4 | | 0 | - | 2 | 0.5 | | 0 | - | 2 | 1.3 | | 0 | - | 2 | 1.7 | |
| Physical activity (5 years—sport) | | | | | 0.6433 | | | | | 0.9701 | | | | | 0.0359 | | | | | 0.0550 |
| 1. > 5 h/week | 2 | 8.0 | 69 | 14.7 | | 10 | 15.4 | 61 | 14.2 | | 57 | 17.0 | 14 | 8.9 | | 61 | 16.4 | 10 | 8.3 | |
| 3. 2–4 h/week | 11 | 44.0 | 190 | 40.5 | | 26 | 40.0 | 175 | 40.8 | | 128 | 38.1 | 73 | 46.2 | | 144 | 38.7 | 57 | 47.1 | |
| 4. <2 h/week | 12 | 48.0 | 208 | 44.3 | | 29 | 44.6 | 191 | 44.5 | | 150 | 44.6 | 70 | 44.3 | | 166 | 44.6 | 53 | 43.8 | |
| Missing | 0 | - | 2 | 0.4 | | 0 | - | 2 | 0.5 | | 1 | 0.3 | 1 | 0.6 | | 1 | 0.3 | 1 | 0.8 | |
| Physical activity (1 year—work) | | | | | 0.6425 | | | | | 0.4306 | | | | | 0.8070 | | | | | 0.8103 |
| 1. (Very) heavy/moderate | 5 | 20.0 | 134 | 28.6 | | 20 | 30.8 | 119 | 27.7 | | 93 | 27.7 | 46 | 29.1 | | 102 | 27.4 | 36 | 29.8 | |
| 3. Mainly standing | 6 | 24.0 | 100 | 21.3 | | 10 | 15.4 | 96 | 22.4 | | 75 | 22.3 | 31 | 19.6 | | 82 | 22.0 | 24 | 19.8 | |
| 4. Mainly sitting | 14 | 56.0 | 233 | 49.7 | | 35 | 53.8 | 212 | 49.4 | | 168 | 50.0 | 79 | 50.0 | | 188 | 50.5 | 59 | 48.8 | |

**Table 1.** *Cont.*

| | **Good Quality Oocytes** | | | | | **Embryo Transfer** | | | | | **Clinical Pregnancy** | | | | | **Pregnancy at Term *** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **No N = 25** | | **Yes N = 469** | | | **No N = 65** | | **Yes N = 429** | | | **No N = 336** | | **Yes N = 158** | | | **No N = 372** | | **Yes N = 121** | | |
| | **N** | **%** | **N** | **%** | ***p*-Value **** | **N** | **%** | **N** | **%** | ***p*-Value **** | **N** | **%** | **N** | **%** | ***p*-Value **** | **N** | **%** | **N** | **%** | ***p*-Value **** |
| Missing | 0 | - | 2 | 0.4 | | 0 | - | 2 | 0.5 | | 0 | - | 2 | 1.3 | | 0 | - | 2 | 1.7 | |
| Physical activity (1 year—sport) | | | | | 0.6632 | | | | | 0.3091 | | | | | 0.0244 | | | | | 0.1535 |
| 1. > 5 h/week | 2 | 8.0 | 49 | 10.4 | | 10 | 15.4 | 41 | 9.6 | | 43 | 12.8 | 8 | 5.1 | | 44 | 11.8 | 7 | 5.8 | |
| 3. 2–4 h/week | 11 | 44.0 | 165 | 35.2 | | 20 | 30.8 | 156 | 36.4 | | 113 | 33.6 | 63 | 39.9 | | 128 | 34.4 | 47 | 38.8 | |
| 4. <2 h/week | 12 | 48.0 | 254 | 54.2 | | 35 | 53.8 | 231 | 53.8 | | 180 | 53.6 | 86 | 54.4 | | 200 | 53.8 | 66 | 54.5 | |
| Missing | . | . | 1 | 0.2 | | . | . | 1 | 0.2 | | . | . | 1 | 0.6 | | . | . | 1 | 0.8 | |
| Alcohol | | | | | 0.9598 | | | | | 0.6220 | | | | | 0.1163 | | | | | 0.3755 |
| 1. no alcohol intake | 6 | 24.0 | 134 | 28.6 | | 16 | 24.6 | 124 | 28.9 | | 92 | 27.4 | 48 | 30.4 | | 102 | 27.4 | 38 | 31.4 | |
| 2. 0.1–2.36 g/day | 6 | 24.0 | 112 | 23.9 | | 14 | 21.5 | 104 | 24.2 | | 72 | 21.4 | 46 | 29.1 | | 84 | 22.6 | 33 | 27.3 | |
| 3. 2.36–5.85 g/day | 7 | 28.0 | 115 | 24.5 | | 16 | 24.6 | 106 | 24.7 | | 91 | 27.1 | 31 | 19.6 | | 98 | 26.3 | 24 | 19.8 | |
| 4. >5.85 g/day | 6 | 24.0 | 108 | 23.0 | | 19 | 29.2 | 95 | 22.1 | | 81 | 24.1 | 33 | 20.9 | | 88 | 23.7 | 26 | 21.5 | |
| Caffeine intake | | | | | 0.3647 | | | | | 0.8563 | | | | | 0.6692 | | | | | 0.2781 |
| 1. 0–81.9 g/week | 10 | 40.0 | 153 | 32.6 | | 21 | 32.3 | 142 | 33.1 | | 107 | 31.8 | 56 | 35.4 | | 119 | 32.0 | 44 | 36.4 | |
| 2. 82.0–179.0 g/week | 10 | 40.0 | 158 | 33.7 | | 24 | 36.9 | 144 | 33.6 | | 118 | 35.1 | 50 | 31.6 | | 134 | 36.0 | 34 | 28.1 | |
| 3. ≥180.0 g/week; | 5 | 20.0 | 158 | 33.7 | | 20 | 30.8 | 143 | 33.3 | | 111 | 33.0 | 52 | 32.9 | | 119 | 32.0 | 43 | 35.5 | |
| Calories (kcal/day) | | | | | 0.9542 | | | | | 0.7854 | | | | | 0.1478 | | | | | 0.3812 |
| 1. 0–1525.7 | 9 | 36.0 | 155 | 33.0 | | 20 | 30.8 | 144 | 33.6 | | 108 | 32.1 | 56 | 35.4 | | 120 | 32.3 | 43 | 35.5 | |
| 2. 1525.8–1923.9 | 8 | 32.0 | 158 | 33.7 | | 21 | 32.3 | 145 | 33.8 | | 107 | 31.8 | 59 | 37.3 | | 122 | 32.8 | 44 | 36.4 | |
| 3. 1924–3344 | 8 | 32.0 | 156 | 33.3 | | 24 | 36.9 | 140 | 32.6 | | 121 | 36.0 | 43 | 27.2 | | 130 | 34.9 | 34 | 28.1 | |
| FSH (mIU/mL) | | | | | 0.1111 | | | | | <0.0001 | | | | | 0.0001 | | | | | <0.0001 |
| <5.80 | 5 | 20.0 | 114 | 24.3 | | 11 | 16.9 | 108 | 25.2 | | 71 | 21.1 | 48 | 30.4 | | 81 | 21.8 | 38 | 31.4 | |
| 5.80–7.29 | 3 | 12.0 | 123 | 26.2 | | 9 | 13.8 | 117 | 27.3 | | 79 | 23.5 | 47 | 29.7 | | 87 | 23.4 | 39 | 32.2 | |
| 7.30–8.89 | 5 | 20.0 | 117 | 24.9 | | 12 | 18.5 | 110 | 25.6 | | 79 | 23.5 | 43 | 27.2 | | 89 | 23.9 | 32 | 26.4 | |
| ≥8.9 | 11 | 44.0 | 115 | 24.5 | | 32 | 49.2 | 94 | 21.9 | | 106 | 31.5 | 20 | 12.7 | | 114 | 30.6 | 12 | 9.9 | |
| Missing | 1 | 4.0 | 0 | - | | 1 | 1.5 | 0 | - | | 1 | 0.3 | 0 | - | | 1 | 0.3 | 0 | - | |
| AMH (ng/mL) | | | | | 0.0010 | | | | | 0.0011 | | | | | <0.0001 | | | | | <0.0001 |
| <0.81- | 12 | 48.0 | 109 | 23.2 | | 26 | 40.0 | 95 | 22.1 | | 100 | 29.8 | 21 | 13.3 | | 108 | 29.0 | 13 | 10.7 | |
| 0.81–1.59 | 8 | 32.0 | 107 | 22.8 | | 17 | 26.2 | 98 | 22.8 | | 81 | 24.1 | 34 | 21.5 | | 88 | 23.7 | 26 | 21.5 | |
| 1.60–3.19 | 3 | 12.0 | 123 | 26.2 | | 10 | 15.4 | 116 | 27.0 | | 85 | 25.3 | 41 | 25.9 | | 92 | 24.7 | 34 | 28.1 | |
| ≥3.20 | 0 | - | 124 | 26.4 | | 8 | 12.3 | 116 | 27.0 | | 65 | 19.3 | 59 | 37.3 | | 77 | 20.7 | 47 | 38.8 | |

**Table 1.** *Cont.*

| | Good Quality Oocytes | | | | | Embryo Transfer | | | | | Clinical Pregnancy | | | | | Pregnancy at Term * | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **No N = 25** | | **Yes N = 469** | | | **No N = 65** | | **Yes N = 429** | | | **No N = 336** | | **Yes N = 158** | | | **No N = 372** | | **Yes N = 121** | | |
| | **N** | **%** | **N** | **%** | ***p*-Value **** | **N** | **%** | **N** | **%** | ***p*-Value **** | **N** | **%** | **N** | **%** | ***p*-Value **** | **N** | **%** | **N** | **%** | ***p*-Value **** |
| Missing | 2 | 8.0 | 6 | 1.3 | | 4 | 6.2 | 4 | 0.9 | | 5 | 1.5 | 3 | 1.9 | | 7 | 1.9 | 1 | 0.8 | |
| Oocytes used (number) | | | | | <0.0001 | | | | | <0.0001 | | | | | <0.0001 | | | | | <0.0001 |
| <3.0 | 25 | 100.0 | 87 | 18.6 | | 49 | 75.4 | 63 | 14.7 | | 103 | 30.7 | 9 | 5.7 | | 106 | 28.5 | 6 | 5.0 | |
| 3.00–4.99 | 0 | - | 121 | 25.8 | | 3 | 4.6 | 118 | 27.5 | | 89 | 26.5 | 32 | 20.3 | | 100 | 26.9 | 21 | 17.4 | |
| 5.00–7.99 | 0 | - | 127 | 27.1 | | 9 | 13.8 | 118 | 27.5 | | 76 | 22.6 | 51 | 32.3 | | 88 | 23.7 | 38 | 31.4 | |
| ≥8.00 | 0 | - | 134 | 28.6 | | 4 | 6.2 | 130 | 30.3 | | 68 | 20.2 | 66 | 41.8 | | 78 | 21.0 | 56 | 46.3 | |

Abbreviations: AMH, anti-Müllerian hormone; BMI, body mass index; FSH, follicle-stimulating hormone; NE, Not evaluable; * one missing ** *p*-value(Chi-square Test).

The mean (SD) age was 36.9 (3.6) years (range 27–46 years). The mean (SD) BMI was 22.4 (4.0) kg/m$^2$ (range 17.0–42.0 kg/m$^2$). Twenty-nine (5.9%) women were obese (BMI > 30 kg/m$^2$). Considering the four IVF outcomes, 469 women (95%) achieved good quality oocytes, 429 women (87%) achieved embryo transfer, 158 women (32.0%) achieved clinical pregnancies, and 121 women (24.5%) achieved pregnancy at term. Associations were found between age and the number of good quality oocytes ($p = 0.0223$). Women aged 35–39 years had a higher number of good quality oocytes and a higher rate of achieving clinical pregnancy and pregnancy at term ($p = 0.0223$ and $p < 0.0001$, respectively). Moderate physical activity in the prior 5 years (2–4 h of sport per week) was associated with a better rate of achieving clinical pregnancy ($p = 0.0359$). Among other lifestyle indicators, smoking habits, alcohol intake, and caffeine consumption did not show associations with any outcome. Descriptive statistics are reported in Table 2.

Adjusted relative risks (ARRs) and 95% CI for vitamins (C, D, E), α-carotene, β-carotene, beta-cryptoxanthin, lutein and folate are reported in Table 3. Considering all four outcomes, and each antioxidant, no associations were found, including after accounting for potential confounders. Higher concentrations of the follicle-stimulating hormone were not associated with the number of good quality oocytes ($p = 0.1111$). Follicle-stimulating hormone concentrations were negatively associated with embryo transfer, clinical pregnancy, and pregnancy at term ($p < 0.0001$).

The Anti-Müllerian hormone (AMH) concentrations and the number of oocytes used for retrieval were associated with all the IVF outcomes considered (all $p < 0.0001$) (Table 4).

**Table 2.** Antioxidant and micronutrient intake of 494 women according to IVF outcomes.

| Variable | Good Quality Oocytes | | | | Embryo Transfer | | | | Clinical Pregnancy | | | | Pregnancy at Term | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No (25) | | Yes (469) | | No (65) | | Yes (429) | | No (336) | | Yes (158) | | No (372) | | Yes (121) | |
| | Median | Q1–Q3 | Median | Q1–Q3 | Median | Q1–Q3 | Median | Q1–Q3 | Median | Q1–Q3 | Median | Q1–Q3 | Median | Q1–Q3 | Median | Q1–Q3 |
| Vitamin C mg/day | 125.4 | 91–167 | 127.7 | 94–167 | 123.6 | 90–168 | 127.8 | 94–167 | 130.0 | 94–172 | 122.7 | 94–157 | 128.4 | 94–171 | 123.8 | 92–153 |
| Vitamin D μg/day | 3.2 | 3–4 | 3.2 | 2–4 | 3.2 | 2–4 | 3.2 | 2–4 | 3.2 | 2–4 | 3.2 | 2–4 | 3.2 | 2–4 | 3.1 | 2–4 |
| Vitamin E mg/day | 11.2 | 10–13 | 10.8 | 9–14 | 11.4 | 10–14 | 10.7 | 9–14 | 10.9 | 9–14 | 10.6 | 8–14 | 10.9 | 9–14 | 10.6 | 8–14 |
| α-carotene μg/day | 763.8 | 537–1246 | 742.5 | 453–1344 | 778.4 | 526–1295 | 732.4 | 447–1344 | 761.4 | 485–1370 | 685.3 | 410–1272 | 758.6 | 469–1342 | 683.9 | 410–1363 |
| β-carotene μg/day | 4356.8 | 3309–5484 | 4170.3 | 3156–5842 | 4224.4 | 3298–6099 | 4170.3 | 3156–5819 | 4389.9 | 3189–5868 | 3966.7 | 3156–5763 | 4348.6 | 3221–5839 | 3869.0 | 3031–5886 |
| β-cryptoxanthin μg/day | 144.6 | 121–288 | 194.9 | 108–291 | 145.2 | 92–277 | 207.4 | 109–292 | 192.2 | 109–293 | 198.1 | 106–288 | 194.1 | 110–293 | 194.1 | 90–287 |
| Lutein μg/day | 355.8 | 269–429 | 308.8 | 235–420 | 314.0 | 236–429 | 310.2 | 235–420 | 308.1 | 236–415 | 314.3 | 224–425 | 308.2 | 234–415 | 315.6 | 240–425 |
| Folate μg/day | 210.7 | 195–239 | 217.9 | 184–259 | 211.7 | 190–258 | 220.3 | 185–258 | 223.2 | 187–262 | 209.0 | 181–249 | 221.1 | 188–261 | 208.3 | 176–248 |

**Table 3.** Adjusted relative risks (ARR) and 95% confidence intervals (CI) for percentiles of selected food intake.

| | Good Quality Oocytes | | | | | Embryo Transfer | | | | | Clinical Pregnancy | | | | | Pregnancy at Term | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable § | Yes | % | ARR * | 95% CI | | Yes | % | ARR ** | 95% CI | | Yes | % | ARR *** | 95% CI | | Yes | % | ARR *** | 95% CI | |
| Vitamin C mg/day | | | | | | | | | | | | | | | | | | | | |
| 1st (29.8–94.1) | 116 | 94.3 | 1+ | | | 104 | 84.6 | 1+ | | | 39 | 31.7 | 1+ | | | 32 | 26.0 | 1+ | | |
| 2nd (94.1–127.6) | 118 | 95.2 | 1.01 | 0.95 | 1.07 | 109 | 87.9 | 1.04 | 0.94 | 1.15 | 46 | 37.1 | 1.17 | 0.83 | 1.65 | 31 | 25.2 | 0.97 | 0.63 | 1.48 |
| 3rd (127.6–167.4) | 118 | 95.9 | 1.02 | 0.96 | 1.08 | 110 | 89.4 | 1.06 | 0.96 | 1.17 | 42 | 34.1 | 1.08 | 0.75 | 1.54 | 36 | 29.3 | 1.12 | 0.75 | 1.69 |
| 4th (167.4–471.2) | 117 | 94.4 | 1.00 | 0.94 | 1.06 | 106 | 85.5 | 1.01 | 0.91 | 1.12 | 31 | 25.0 | 0.79 | 0.53 | 1.18 | 22 | 17.7 | 0.68 | 0.42 | 1.10 |
| Vitamin D μg/day | | | | | | | | | | | | | | | | | | | | |
| 1st (0.3–2.3) | 118 | 95.9 | 1+ | | | 106 | 86.2 | 1+ | | | 35 | 28.5 | 1+ | | | 28 | 22.8 | 1+ | | |
| 2nd (2.3–3.2) | 117 | 94.4 | 0.98 | 0.93 | 1.04 | 109 | 87.9 | 1.02 | 0.93 | 1.12 | 43 | 34.7 | 1.22 | 0.84 | 1.76 | 33 | 26.6 | 1.17 | 0.75 | 1.81 |
| 3rd (3.2–4.0) | 118 | 95.9 | 1.00 | 0.95 | 1.05 | 109 | 88.6 | 1.03 | 0.94 | 1.13 | 36 | 29.3 | 1.03 | 0.69 | 1.52 | 27 | 22.1 | 0.97 | 0.61 | 1.55 |
| 4th (4.0–9.5) | 116 | 93.5 | 0.98 | 0.92 | 1.03 | 105 | 84.7 | 0.98 | 0.89 | 1.09 | 44 | 35.5 | 1.25 | 0.86 | 1.80 | 33 | 26.6 | 1.17 | 0.75 | 1.81 |
| Vitamin E mg/day | | | | | | | | | | | | | | | | | | | | |
| 1st (3.9–8.6) | 119 | 96.7 | 1+ | | | 108 | 87.8 | 1+ | | | 45 | 36.6 | 1+ | | | 36 | 29.5 | 1+ | | |
| 2nd (8.6–10.9) | 119 | 96.0 | 0.99 | 0.94 | 1.04 | 113 | 91.1 | 1.04 | 0.95 | 1.13 | 36 | 29.0 | 0.79 | 0.55 | 1.14 | 26 | 21.0 | 0.71 | 0.46 | 1.10 |
| 3rd (10.9–13.7) | 112 | 91.1 | 0.94 | 0.88 | 1.00 | 100 | 81.3 | 0.93 | 0.83 | 1.03 | 37 | 30.1 | 0.82 | 0.58 | 1.17 | 28 | 22.8 | 0.77 | 0.50 | 1.18 |
| 4th (13.7–30.0) | 119 | 96.0 | 0.99 | 0.94 | 1.04 | 108 | 87.1 | 0.99 | 0.90 | 1.09 | 40 | 32.3 | 0.88 | 0.62 | 1.25 | 31 | 25.0 | 0.85 | 0.56 | 1.28 |

**Table 3.** *Cont.*

| | Good Quality Oocytes | | | | | Embryo Transfer | | | | | Clinical Pregnancy | | | | | Pregnancy at Term | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** [§] | **Yes** | **%** | **ARR *** | **95% CI** | | **Yes** | **%** | **ARR **** | **95% CI** | | **Yes** | **%** | **ARR ***** | **95% CI** | | **Yes** | **%** | **ARR ***** | **95% CI** | |
| α-carotene μg/day | | | | | | | | | | | | | | | | | | | | |
| 1st (17.9–458.6) | 118 | 95.9 | 1+ | | | 109 | 88.6 | 1+ | | | 44 | 35.8 | 1+ | | | 33 | 26.8 | 1+ | | |
| 2nd(458.6–742.6) | 117 | 94.4 | 0.98 | 0.93 | 1.04 | 109 | 87.9 | 0.99 | 0.91 | 1.09 | 41 | 33.1 | 0.92 | 0.65 | 1.30 | 32 | 26.0 | 0.97 | 0.64 | 1.47 |
| 3rd (742.6–1344.2) | 117 | 94.4 | 0.98 | 0.93 | 1.04 | 104 | 83.9 | 0.95 | 0.86 | 1.05 | 37 | 29.8 | 0.83 | 0.58 | 1.19 | 25 | 20.2 | 0.75 | 0.48 | 1.19 |
| 4th (1344.2–5604.7) | 117 | 95.1 | 0.99 | 0.94 | 1.05 | 107 | 87.0 | 0.98 | 0.89 | 1.08 | 36 | 29.3 | 0.82 | 0.57 | 1.18 | 31 | 25.2 | 0.94 | 0.62 | 1.43 |
| β-carotene μg/day | | | | | | | | | | | | | | | | | | | | |
| 1st (613.2–3168.8) | 118 | 95.9 | 1+ | | | 109 | 88.6 | 1+ | | | 41 | 33.3 | 1+ | | | 34 | 27.6 | 1+ | | |
| 2nd (3168.8–4194.6) | 117 | 94.4 | 0.98 | 0.93 | 1.04 | 106 | 85.5 | 0.96 | 0.88 | 1.06 | 47 | 37.9 | 1.14 | 0.81 | 1.59 | 34 | 27.6 | 1.00 | 0.67 | 1.50 |
| 3rd (4194.6–5841.5) | 116 | 94.3 | 0.98 | 0.93 | 1.04 | 108 | 87.8 | 0.99 | 0.90 | 1.09 | 31 | 25.2 | 0.76 | 0.51 | 1.12 | 22 | 17.9 | 0.65 | 0.40 | 1.04 |
| 4th (5841.5–17908.9) | 118 | 95.2 | 0.99 | 0.94 | 1.05 | 106 | 85.5 | 0.96 | 0.88 | 1.06 | 39 | 31.5 | 0.94 | 0.66 | 1.35 | 31 | 25.0 | 0.90 | 0.60 | 1.37 |
| β-cryptoxanthin μg/day | | | | | | | | | | | | | | | | | | | | |
| 1st (1.0–108.0) | 117 | 95.1 | 1+ | | | 105 | 85.4 | 1+ | | | 41 | 33.3 | 1+ | | | 33 | 26.8 | 1+ | | |
| 2nd (108.0–194.1) | 116 | 93.5 | 0.98 | 0.93 | 1.05 | 99 | 79.8 | 0.94 | 0.83 | 1.05 | 36 | 29.0 | 0.87 | 0.60 | 1.26 | 28 | 22.6 | 0.84 | 0.54 | 1.30 |
| 3rd (194.1–290.9) | 118 | 95.9 | 1.01 | 0.96 | 1.06 | 113 | 91.9 | 1.08 | 0.98 | 1.18 | 48 | 39.0 | 1.17 | 0.84 | 1.63 | 39 | 32.0 | 1.19 | 0.81 | 1.76 |
| 4th (290.9–1240.2) | 118 | 95.2 | 1.00 | 0.95 | 1.06 | 112 | 90.3 | 1.06 | 0.96 | 1.16 | 33 | 26.6 | 0.80 | 0.54 | 1.17 | 21 | 16.9 | 0.63 | 0.39 | 1.03 |
| Lutein μg/day | | | | | | | | | | | | | | | | | | | | |
| 1st (72.0–234.8) | 118 | 95.2 | 1+ | | | 108 | 87.1 | 1+ | | | 41 | 33.1 | 1+ | | | 29 | 23.6 | 1+ | | |
| 2nd (234.8–311.1) | 119 | 96.7 | 1.02 | 0.97 | 1.07 | 108 | 87.8 | 1.01 | 0.92 | 1.11 | 33 | 26.8 | 0.81 | 0.55 | 1.19 | 27 | 22.0 | 0.93 | 0.59 | 1.48 |
| 3rd (311.1–420.3) | 116 | 93.5 | 0.98 | 0.92 | 1.04 | 107 | 86.3 | 0.99 | 0.90 | 1.09 | 43 | 34.7 | 1.05 | 0.74 | 1.49 | 33 | 26.6 | 1.13 | 0.73 | 1.74 |
| 4th (420.3–945.0) | 116 | 94.3 | 0.99 | 0.93 | 1.05 | 106 | 86.2 | 0.99 | 0.90 | 1.09 | 41 | 33.3 | 1.01 | 0.71 | 1.44 | 32 | 26.0 | 1.10 | 0.71 | 1.71 |
| Folate μg/day | | | | | | | | | | | | | | | | | | | | |
| 1st (76.5–185.4) | 118 | 95.9 | 1+ | | | 107 | 87.0 | 1+ | | | 44 | 35.8 | 1+ | | | 36 | 29.5 | 1+ | | |
| 2nd (185.4–217.6) | 113 | 91.1 | 0.95 | 0.89 | 1.01 | 101 | 81.5 | 0.94 | 0.84 | 1.04 | 46 | 37.1 | 1.04 | 0.75 | 1.44 | 32 | 25.8 | 0.87 | 0.58 | 1.31 |
| 3rd (217.6–258.1) | 119 | 96.7 | 1.01 | 0.96 | 1.06 | 113 | 91.9 | 1.06 | 0.97 | 1.15 | 36 | 29.3 | 0.82 | 0.57 | 1.18 | 28 | 22.8 | 0.77 | 0.50 | 1.18 |
| 4th (258.1–434.3) | 119 | 96.0 | 1.00 | 0.95 | 1.05 | 108 | 87.1 | 1.00 | 0.91 | 1.10 | 32 | 25.8 | 0.72 | 0.49 | 1.06 | 25 | 20.2 | 0.68 | 0.44 | 1.07 |

[§] The cut-off points of the quartiles of daily intake for the micronutrients are expressed in brackets.First reference category for all variables. * Adjusted for age. ** Adjusted for age and BMI. *** Adjusted for age and prior 5 years of leisure physical activity.

**Table 4.** Concentrations of Anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH) and number of oocytes used for retrieval according to IVF outcomes.

| **Variable** | **Good Quality Oocytes** | | | | | **Embryo Transfer** | | | | | **Clinical Pregnancy** | | | | | **Pregnancy at Term** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **No (25)** | | **Yes (469)** | | | **No (65)** | | **Yes (429)** | | | **No (336)** | | **Yes (158)** | | | **No (372)** | | **Yes (121)** | | |
| | **Median** | **Q1–Q3** | **Median** | **Q1–Q3** | ***p**** | **Median** | **Q1-Q3** | **Median** | **Q1-Q3** | ***p**** | **Median** | **Q1–Q3** | **Median** | **Q1-Q3** | ***p**** | **Median** | **Q1–Q3** | **Median** | **Q1–Q3** | ***p**** |
| FSH (mIU/mL) | 8.6 | 6.4–11.5 | 7.2 | 5.8–8.8 | 0.0241 | 8.9 | 6.8–11.1 | 7.0 | 5.7–8.6 | <0.0001 | 7.8 | 6.1–9.8 | 6.7 | 5.4–8.1 | <0.0001 | 7.6 | 6.0–9.6 | 6.5 | 5.3–7.9 | <0.0001 |
| AMH (ng/mL) | 0.8 | 0.3–1.4 | 1.7 | 0.9–3.3 | <0.0001 | 1.0 | 0.4–1.9 | 1.7 | 0.9–3.4 | <0.0001 | 1.4 | 0.7–2.8 | 2.2 | 1.2–3.9 | <0.0001 | 1.5 | 0.7–2.8 | 2.3 | 1.2–3.9 | <0.0001 |
| Number of oocytes used | 0.0 | 0.0–0.0 | 5.0 | 3.0–8.0 | <0.0001 | 1.0 | 0.0–2.0 | 5.0 | 3.0–8.0 | <0.0001 | 4.0 | 2.0–7.0 | 7.0 | 4.0–9.0 | <0.0001 | 4.0 | 2.0–7.0 | 7.0 | 5.0–10.0 | <0.0001 |

* 1-sided non-parametric Wilcoxon test.

## 4. Discussion

In a cohort of Italian women, we examined whether the intake of vitamins C, D, E, and α-carotene, β-carotene, beta-cryptoxanthin, lutein, and folate is associated with four outcomes of ART. Our results do not find any associations between these micronutrients and any of the IVF outcomes considered. We find evidence of association between a moderate level of physical activity in the prior 5 years and a better rate of achieving clinical pregnancy. No associations emerge with the other lifestyle indicators. It is well demonstrated how lifestyle factors affect fertility, and inappropriate diet or insufficient dietary micronutrients may manifest itself in subfertility. Similarly to our results on physical activity, Chavarro et al. showed that a combination of adequate diet, physical activity, and weight control was associated with a 69% lower risk of subfertility due to ovulatory disorders [23].

Micronutrients play independent roles for the development of the fetus and the prevention of pregnancy-related complications [24]. There is biological plausibility to hypothesize that these micronutrients, sharing anti-inflammatory activities, may be helpful in infertility treatment. The intensive metabolism of granulosa cells and the high numbers of macrophages and neutrophilic granulocytes in the follicle wall at ovulation may point to active generation of Reactive Oxygen Species (ROS). ROS levels, within certain physiological ranges, may be necessary for the normal development of the oocyte and embryo growth; however, high levels may indicate OS. Animal studies have demonstrated that deficiencies of vitamins A, C, and D result in diminished fertility [25,26]. Studies of men indicate that diet is crucial in preventing oxidative damage to sperm DNA [27]. Furthermore, antioxidants have been shown to be associated with reproductive hormone concentrations in healthy women, suggesting potentially complex hormonal interactions [28].

In results similar to ours, Ming-Chieh Li et al. [13] followed 349 women undergoing ART cycles for infertility treatment. The authors found that the total consumption of vitamins A, C, and E prior to starting infertility treatment with ART was not associated with live birth rates. Other results raised unexpected inverse associations of β-carotene intake from foods and of lutein with live birth rates. Conversely, Ruder et al. reported that a higher intake of β-carotene was related to a longer time to pregnancy among women over 35 years who participated in an RCT of infertility treatment, and the opposite relation was observed among younger women [11]. Moreover, a prospective cohort study of 1228 women measured preconception levels of, among other variables, cryptoxanthin, α- and β-carotene, and α- and γ-tocopherol in serum. The authors found that higher preconception serum carotenoid concentrations were associated with improved fecundability and a shorter time to pregnancy among women with no history of infertility. By contrast, serum γ-tocopherol levels that were at or above the US average were associated with a longer time to pregnancy. A positive association between carotenoids and shortened time to pregnancy was also reported in a recent study of women diagnosed with unexplained infertility [11]. Regarding the folate, supplemental folate, at intake levels that are much higher than those currently recommended, was related to a higher probability of live birth among women undergoing ART [29].

Most of the literature in this field concerns the investigation of the effect of supplementation with mixtures of antioxidants or other supplements that include antioxidants; however, epidemiologic studies and RCTs focusing on single antioxidants, including vitamins A, C, and E, or carotenoids, are limited and produced inconsistent findings. A 2017 Cochrane review of RCTs concluded that among women undergoing ART, antioxidants were not associated with an increased live birth rate, and this review did not draw strong conclusions regarding the effects of supplementation of antioxidants on ART outcomes [8].

The heterogeneity of the FFQ used, as well as the inter-variability of food composition tables, which may even differ year to year, may at least partly explain these differing findings [30].

Clear dietary recommendations have not yet been established for preconception health, except for the assumption of 400 mcg/day of folic acid, to prevent defects of the neural tube development. Specific supplements, except for folic acid, are not routinely advocated by gynecologists before IVF. Consequently, during the study period, supplements were not routinely prescribed in our clinical center. Furthermore, the interest of the lay press in the role of supplements in improving fertility has only increased more recently; thus, it is conceivable that only a very limited proportion of women considered in this study were using supplements. Some surveys have shown that healthier dietary patterns, characterized by better adherence to the Mediterranean diet—with high consumption of legumes, whole grains, vegetables, and fruits (therefore rich in vitamins C, D, E, as well as α-carotene, β-carotene, beta-cryptoxanthin, lutein, and folate)—and lower intakes of both processed foods and sugar-sweetened beverages, are associated with shorter time to pregnancy, higher fecundability, higher rates of clinical pregnancy and live birth, and a reduced risk of ovulatory disorder infertility [31–33].

A strength of our report is represented by its prospective design and the high participation rate in our relatively large sample size in a monocentric survey. However, some limitations should be noted. First, our clinical center, at the time of the study, did not advise the use of antioxidants or other supplements except for folic acid, and we did not collect any information on the type of supplement that the women were using prior to the IVF treatment. Consequently, all of our results on the consumption of micronutrients are related to the micronutrients derived from food sources. Second, measurement bias was inevitable, and it may have occurred because participants may underestimate or overestimate their dietary intakes. Finally, these results may not be generalizable to women without known fertility problems.

## 5. Conclusions

Subfertile women are highly motivated to explore all paths of treatment in their desire to have a healthy baby. Antioxidants are mostly unregulated and are readily available for purchase by consumers. Women should therefore be made more aware of the role of lifestyle changes and an overall healthy diet. Reliable knowledge about the role of nutrition on fertility is far from complete, and more long-term investigations with larger cohorts are recommended to confirm our findings. However, up to the availability of robust interventional evidence, our results argue against the use of antioxidants in women requiring IVF.

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**Informed Consent Statement:** Written informed consent has been obtained from the patients to publish this paper.

**Data Availability Statement:** The raw data supporting the conclusions article will be made available by the authors without undue reservation.

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