

## **Food supplements in the treatment of male infertility: a critical review on their formulations and use**

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## Abstract

**Background:** Dietary supplements (DS) represent a possible approach to improve sperm parameters and male fertility. A wide range of DS containing different nutrients is now available. Although many authors demonstrated benefits from some nutrients in male infertility, their real effectiveness is still under debate. The aim of this study was to critically review the composition of DS using the Italian market as sample.

**Materials & Methods:** Active ingredients and their minimal effective daily dose (mFED) were identified through literature search. Thereafter, we created a formula to classify the expected efficacy of each DS. Considering active ingredients, their concentration and the recommended daily dose, DS were scored into three classes of expected efficacy: higher, lower and none.

**Results:** Twenty-one DS were identified. Most of them had a large number of ingredients, frequently at doses below mFED or with unproven efficacy. Zinc was the most common ingredient of DS (70% of products), followed by selenium, arginine, coenzyme Q and folic acid. By applying our scoring system, 9.5% of DS fell in higher class, 71.4% in lower class and 19.1% in the class with no expected efficacy.

**Conclusions:** DS marketed in Italy for male infertility frequently include effective ingredients but also a large number of substances at insufficient dose or with no proven efficacy. Manufacturers and physicians should better consider the scientific evidence on effective ingredients and their doses before formulating and prescribing these products.

**Keywords:** fertility; male reproduction; semen parameters; supplements; ingredients

## Introduction

Infertility is a pathological condition defined as the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year [1]. Both male and female factors can lead to infertility. In particular, according to the causes, it has been reported that 29.3% is due to male a factor, 37.1% to a female factor, 17.6% to both male and female factors, with the remaining percentage considered as idiopathic [2].

It is estimated that around 10-15% of all couples are affected by infertility, thus representing a global concern in most of developed countries [3].

Among male infertility causes, many recent studies have emphasized the role of genital tract inflammation, incorrect lifestyles and malnutrition [4]. On this regard, weight excess and other conditions such as metabolic syndrome, alcohol abuse, cigarette smoking, exposure to environmental pollutants etc. have been strongly related to worse sperm quality and infertility. A major driving hypothesis is that these conditions, by inducing an elevation of reactive oxygen species (ROS) and nitrogen species (RONS), are able to alter the balance of the redox status of both the steroidogenic cell population and the germ line cell populations, leading to the impairment of the hypothalamic-pituitary-testicular axis and the reduction of sperm quality [5].

A large number of recent studies have focused on the ability of many substances, generally termed as *nutraceuticals*, to improve the hormonal status and sperm parameters by different mechanisms [6]. Nutraceuticals are used as ingredients of dietary supplements (DS), widely marketed for the prevention or treatment of the most disparate pathological conditions. From a legislative point of view, the European Food Safety Agency (EFSA) defines that DS are not intended for the treatment or prevention of disease in humans, but only to support specific physiological function [7]. Currently, DS are widely prescribed to improve physiological aspects related to male fertility.

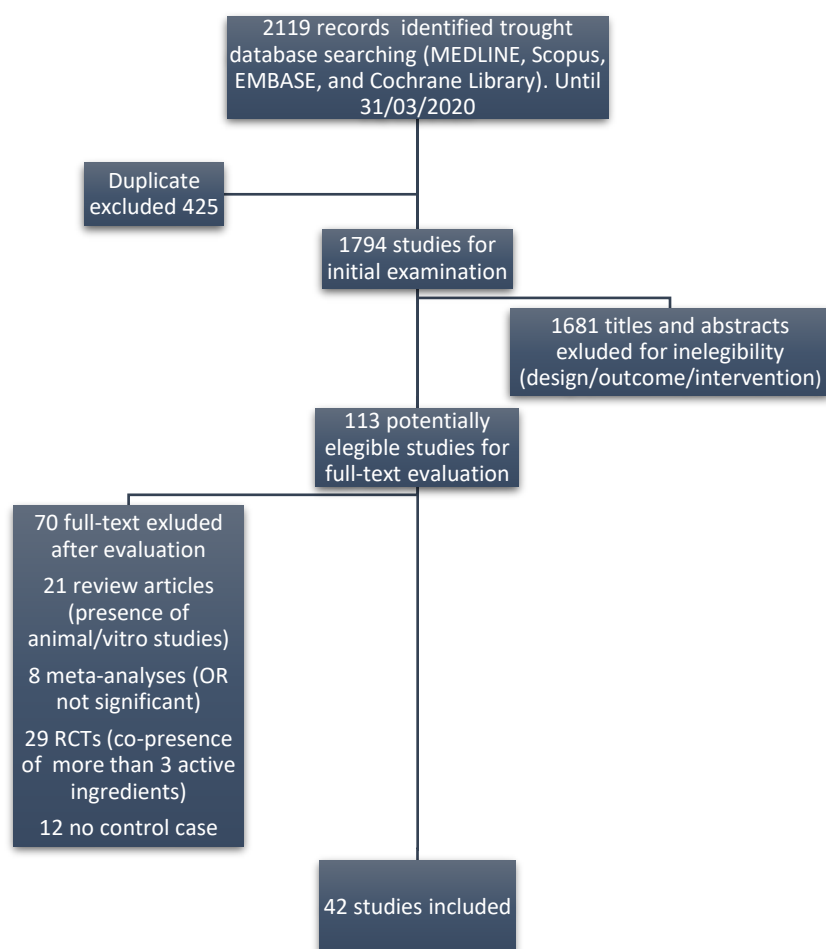
Many DS are available on the market with various formulations, containing both nutrients and botanicals at different doses. Despite many authors have demonstrated positive effects of some ingredients on semen parameters and fertility outcomes [8], many others also showed the lack of efficacy and even potentially harmful side effects [9]. In a recent position statement, the Italian Society of Andrology and Sexual Medicine (SIAMS) summarized the state of the art on each single ingredient currently used in the andrological field. In this paper authors concluded that there is still limited scientific evidence on the possible role of any nutraceutical in andrology and that the use of antioxidants can be suggested only in patients with idiopathic infertility, after a specific diagnostic workup. However, to date no regulation or guidelines are available for the use of these products, generating confusion for both prescribers and patients [10]. Moreover, several factors make difficult and still empirical to address the right ingredient for the right patient. In particular, it is difficult to identify the correct DS since each product contains different ingredients ad different doses.

The purpose of this study was to evaluate the likely efficacy of the DS typically used for male infertility, estimated on base of the declared composition and on available data on their safety-activity profile, using the Italian market as sample.

## Materials & Methods

We collected the names and formulations of the products registered in Italy by referring to the register of the Italian Ministry of Health [11].

A systematic literature review was also performed in order to evaluate, for all the supplements identified, the effectiveness of their ingredients in improving male fertility and their minimum effective dose per day. The literature search was conducted in MEDLINE, Scopus, EMBASE, and Cochrane Library registers until 31/03/2020. The key terms used for the search were: fertility or male reproduction or semen parameters and supplements or ingredients. Only randomized clinical trials (RCT), meta-analyses and reviews were considered eligible. In order to rule out the possible interactions between ingredients, only studies that used active substances alone or in combination with at most other three ingredients were considered. Figure 1 displays the flow diagram of the selection of eligible papers.



**Figure 1.** Flow diagram of the selection of eligible papers.

To establish the efficacy of each ingredients we considered only dose having at least one RCT or systematic review and metaanalysis of RCTs, demonstrating a significant effect on any sperm parameters involved in male fertility. Significance was set at p-value <0.05. Regarding the daily dose of each active ingredient with nutrient characteristics, we referred to the tolerable Upper intake Levels (UL) as reported in Dietary Reference Intake (DRI) [12].

Based on the results of available articles, we were able to identify the minimal effective daily dose (mFED) able to improve male fertility for each active ingredient. Therefore, we classified ingredients and suggested daily dose in each supplement into three categories (A,B,C): reported efficacy with a dose achieving the mFED (A), reported efficacy but with a dose below mFED (B) and unreported data of efficacy (C). To classify DS, we created a formula tacking into consideration the three classes of ingredients and their number:

$$Score = \frac{(2A + B - C)}{2N} \times (A + B/2)$$

In particular, the above formula was conceived based on the following sequential steps:

- 1) In particular, each class of ingredients was given an arbitrary value: A = +2, B= +1 and C= -1;
- 2) these values were multiplied for the respective number of ingredients within each supplement (A, B and C respectively), obtaining a total score given by the sum of each category (2A+B-C);
- 3) As the number of ingredients highly differed between supplements, we standardized the above total score by dividing it for the maximum possible score for that supplement, by assuming that each ingredient was of class A (=2N, where N is the total number of ingredients in each supplement);
- 4) In order to correct this value for the number of ingredients of only categories A and B, the relative score was multiplied for the sum of high efficacy ingredients plus half (as a proxy of their lower efficacy) the number of moderate efficacy ingredients (A+B/2), finally obtaining a corrected score for each supplement.

- 5) Given the distribution of the scores resulted into 3 main clusters, we classified DS into three categories, resembling the efficacy of the ingredients: higher expected efficacy (corrected score  $\geq 4$ ), lower expected efficacy ( $4 <$  corrected score  $> 1$ ) and no expected efficacy (corrected score  $\leq 1$ ).

## Results

We evaluated the 21 DS marketed in Italy for male infertility. Within the active ingredients contained in the DS, the literature search allowed us to identify 42 studies (RCTs or meta-analyses) reporting their efficacy on sperm parameters (figure 1). By this analysis we found that 18 of these ingredients had a proven efficacy. The complete list of ingredients with clinical evidence of efficacy, the respective references, evaluated sperm parameters and employed daily doses, are summarized in table 1. In the right column, the mFED of each ingredient is reported. In some RCT and meta-analyses, marked with an asterisk, the employed dose exceeded the reported UL. In particular, all the studies involving zinc evaluated the effect of this ingredient at a dose exceeding UL. For each active ingredient, the evidence of efficacy was supported by at least two RCTs or meta-analysis, excluding astaxanthin, D-aspartic acid and L-citrulline, which had only one reference.

| Active ingredients    | References              | Evaluated Sperm Parameters                                    | Employed daily dose                        | Minimal fertility effective dose (mFED) |
|-----------------------|-------------------------|---|--|---|
| Zinc                  | [23]*<br>[24]*<br>[25]* | concentration<br>concentration<br>morphology                  | 50 mg<br>66 mg<br>66 mg                    | 50 mg                                   |
| Selenium              | [23]<br>[26]<br>[27]    | linear progression<br>concentration<br>concentration/motility | 50 $\mu$ g<br>100 $\mu$ g<br>200 $\mu$ g   | 50 $\mu$ g                              |
| Vitamin B12           | [28]<br>[29]<br>[30]    | count<br>count<br>count                                       | 25 $\mu$ g<br>1500 $\mu$ g<br>6000 $\mu$ g | 25 $\mu$ g                              |
| Folic Acid            | [31]<br>[32]<br>[33]    | count/motility<br>volume/motility<br>DNA damage               | 400 $\mu$ g<br>500 $\mu$ g<br>500 $\mu$ g  | 400 $\mu$ g                             |
| L-Arginine            | [34]<br>[35]            | progressive motility<br>concentration/motility                | 1,4 g<br>1,4 g                             | 1,4 g                                   |
| L-Citrulline          | [36]                    | volume/concentration<br>motility/vitality                     | 1,2 g                                      | 1,2 g                                   |
| $\alpha$ -Lipoic Acid | [37]                    | concentration/motility  | 600 mg                                     | 600 mg                                  |

|                            |                               |  |   |            |
|----------------------------|-------------------------------|--|---|------------|
|                            | [38]<br>[39]                  | count/motility<br>motility/morphology  | 600 mg<br>600 mg                        |            |
| L-Carnitine<br>(LC/LAC)    | [40]<br>[41]<br>[42]<br>[43]  | motility<br>count/motility<br>concentration/motility<br>motility                     | 1 g<br>2 g<br>2 g<br>3 g                | 1 g        |
| N-Acetyl Cysteine<br>(NAC) | [44]<br>[45]<br>[27]          | motility/DNA damage<br>motility/DNA damage<br>motility/morphology                    | 600 mg<br>600 mg<br>600 mg              | 600 mg     |
| Coenzyme Q10               | [46]<br>[47]<br>[48]          | motility<br>count/motility<br>concentration/morphology                               | 200 mg<br>200 mg<br>300 mg              | 200 mg     |
| Astaxanthin                | [49]                          | motility   | 16 mg                                   | 16 mg      |
| D-Aspartic Acid (DAA)      | [50]                          | count/motility   | 2,7 g                                   | 2,7 g      |
| Tribulus Terrestris DE     | [51]<br>[52]<br>[53]          | count/motility<br>morphology/motility<br>count/morphology                            | 250 mg<br>500 mg<br>6000 mg             | 250 mg     |
| Myoinositol                | [54]<br>[55]                  | motility<br>concentration  | 2 g<br>4 g                              | 2 g        |
| $\alpha$ -Tocopherol       | [56]<br>[43]<br>[57]<br>[58]* | motility/DNA damage<br>motility/morphology<br>motility/lipid oxidation<br>DNA damage | 20 mg<br>268.46 mg<br>300 mg<br>1000 mg | 20 mg      |
| Vitamin C                  | [59]<br>[60]<br>[58]          | concentration/motility<br>motility<br>DNA damage                                     | 0.5 g<br>1 g<br>1 g                     | 0.5 g      |
| EPA + DHA<br>EPA + DHA     | [61]<br>[62]                  | concentration/motility<br>DNA damage   | 0.72 g + 0.48 g<br>0.14 g + 1 g         | DHA 0,48 g |
| Lycopene                   | [63]<br>[64]                  | concentration/motility<br>count/morphology   | 4 mg<br>8 mg                            | 4 mg       |

**Table 1.** Active ingredients with evidence of efficacy, references, evaluated sperm parameters, employed daily doses and minimal fertility effective dose (mFED). \*The employed dose exceeded/reach UL.

**LC:** L-Carnitine; **LAC:** Acetyl L-Carnitine; **EPA:** Eicosapentaenoic acid; **DE:** Dry Extract; **DHA:** docosahexaenoic acid

Ingredients without clinical evidence in the improvement of male fertility (no RCT or metanalysis) are listed in table 2.

|                      |
|----------------------|
| Astragalus DE        |
| Damiana DE           |
| Nettle DE            |
| Catuba DE            |
| Ecklonia bicyclis DE |
| L-Taurine            |
| Glutathione          |
| Glucosamine          |
| SOD                  |
| Vitamin D3           |



|            |
|------------|
| Vitamin B1 |
| Riboflavin |
| Niacin     |
| Vitamin B5 |
| Vitamin B6 |
| Biotin     |
| Manganese  |

**Table 2.** Ingredients without clinical evidence of efficacy.**DE:** Dry Extract; **SOD:** super oxide dismutase.








These ingredients were frequently present in analyzed supplements. A large number of DS contained one or more ineffective ingredients. The composition of the 21 DS and the daily doses of their active ingredients are summarized in table 3.

| Active ingredients      | DS 1 😐     |    | DS 2 😊     |    | DS 3 😐     |    | DS 4 😞     |    | DS 5 😐     |    | DS 6 😐     |    | DS 7 😐     |    |
|-------------------------|------------|----|------------|----|------------|----|------------|----|------------|----|------------|----|------------|----|
|                         | S = 3,12   |    | S = 2,08   |    | S = 3,66   |    | S = 0,16   |    | S = 2,1    |    | S = 2,25   |    | S = 3,37   |    |
|                         | Daily dose | EV | Daily dose | EV | Daily dose | EV | Daily Dose | EV | Daily dose | EV | Daily Dose | EV | Daily dose | EV |
| Zinc                    | 7,5 mg     | ○  | 10 mg      | ○  | 12,5 mg    | ○  | 1,5 mg     | ○  |            |    | 13 mg      | ○  |            |    |
| Selenium                | 60 µg      | ●  |            |    | 83 µg      | ●  |            |    |            |    | 30 µg      | ○  | 55 µg      | ●  |
| Vitamin B12             |            |    |            |    |            |    | 33 mg      | ●  |            |    |            |    |            |    |
| Folic Acid              | 200 µg     | ○  |            |    |            |    | 400 µg     | ●  |            |    | 400 µg     | ●  | 200 µg     | ○  |
| L-Arginine              | 100 mg     | ○  |            |    | 1000 mg    | ○  |            |    | 2500 mg    | ●  | 125 mg     | ○  | 30 mg      | ○  |
| L-Citrulline            |            |    |            |    |            |    |            |    |            |    |            |    |            |    |
| α-Lipoic Acid           |            |    |            |    |            |    | 50 mg      | ○  |            |    |            |    |            |    |
| L-Carnitine             |            |    |            |    | 1000 mg    | ●  |            |    |            |    | 200 mg     | ○  | 30 mg      | ○  |
| N-Acetyl Cysteine (NAC) |            |    |            |    |            |    |            |    |            |    |            |    |            |    |
| Coenzyme Q10            | 10 mg      | ○  | 200 mg     | ●  | 10 mg      | ○  |            |    | 200 mg     | ●  | 7,5 mg     | ○  |            |    |
| Astaxanthin             | 15 mg      | ○  |            |    |            |    |            |    |            |    |            |    |            |    |

|                        |       |   |         |   |        |   |  |        |   |        |   |       |   |  |       |         |   |  |  |
|------------------------|-------|---|---------|---|--------|---|--|--------|---|--------|---|-------|---|--|-------|---------|---|--|--|
| D-Aspartic Acid (DAA)  |       |   | 2660 mg | • |        |   |  |        |   |        |   |       |   |  |       |         |   |  |  |
| Tribulus terrestris DE |       |   |         |   | 800 mg | • |  |        |   |        |   |       |   |  |       |         |   |  |  |
| Myoinositol            |       |   |         |   |        |   |  |        |   |        |   |       |   |  |       | 1000 mg | • |  |  |
| α-Tocopherol           | 30 mg | • |         |   | 12 mg  | ○ |  |        |   | 30 mg  | • | 36 mg | • |  | 30 mg | •       |   |  |  |
| Vitamin C              | 60 mg | ○ |         |   |        |   |  |        |   | 180 mg | ○ |       |   |  |       |         |   |  |  |
| DHA                    |       |   |         |   |        |   |  |        |   |        |   |       |   |  |       |         |   |  |  |
| Lycopene               |       |   |         |   | 15 mg  | • |  |        |   |        |   |       |   |  |       |         |   |  |  |
| Astragalus DE          |       |   |         |   | 300 mg | □ |  |        |   |        |   |       |   |  |       |         |   |  |  |
| Damiana DE             |       |   |         |   |        |   |  |        |   |        |   |       |   |  |       |         |   |  |  |
| Nettle DE              |       |   |         |   |        |   |  |        |   |        |   |       |   |  |       |         |   |  |  |
| Catuba DE              |       |   |         |   |        |   |  |        |   |        |   |       |   |  |       |         |   |  |  |
| Ecklonia bicyclis DE   |       |   |         |   |        |   |  |        |   |        |   |       |   |  |       |         |   |  |  |
| L-Taurine              |       |   |         |   |        |   |  |        |   | 500 mg | □ |       |   |  |       |         |   |  |  |
| Glutathione            |       |   |         |   |        |   |  | 30 mg  | □ |        |   | 40 mg | □ |  |       |         |   |  |  |
| Glucosamine            |       |   |         |   |        |   |  |        |   |        |   |       |   |  |       |         |   |  |  |
| SOD                    |       |   |         |   |        |   |  | 154 UI | □ |        |   |       |   |  |       |         |   |  |  |
| Vitamin D3             |       |   |         |   |        |   |  |        |   |        |   |       |   |  |       |         |   |  |  |
| Vitamin B1             |       |   |         |   |        |   |  |        |   |        |   |       |   |  |       |         |   |  |  |
| Riboflavin             |       |   |         |   |        |   |  | 25 mg  | □ |        |   |       |   |  |       |         |   |  |  |
| Niacin                 |       |   |         |   |        |   |  | 36 mg  | □ |        |   |       |   |  |       |         |   |  |  |
| Vitamin B5             |       |   |         |   |        |   |  |        |   |        |   |       |   |  |       |         |   |  |  |
| Vitamin B6             |       |   |         |   |        |   |  | 9,5 mg | □ |        |   |       |   |  |       |         |   |  |  |
| Biotin                 |       |   |         |   |        |   |  |        |   |        |   |       |   |  |       |         |   |  |  |
| Manganese              |       |   |         |   |        |   |  |        |   |        |   |       |   |  |       |         |   |  |  |

| Active ingredients | DS 8 ☹️    |    | DS 9 😊     |    | DS 10 😐    |    | DS 11 ☹️   |    | DS 12 😐    |    | DS 13 😊    |    | DS 14 😐    |    |
|--------------------|------------|----|------------|----|------------|----|------------|----|------------|----|------------|----|------------|----|
|                    | S = 1      |    | S = 2,81   |    | S = 2,66   |    | S = 0,5    |    | S = 1,14   |    | S = 4,32   |    | S = 2,5    |    |
|                    | Daily dose | EV | Daily dose | EV | Daily dose | EV | Daily dose | EV | Daily dose | EV | Daily dose | EV | Daily dose | EV |
| Zinc               |            |    | 40 mg      | ○  | 12,5 mg    | ○  |            |    | 10 mg      | ○  |            |    | 15 mg      | ○  |
| Selenium           |            |    | 60 µg      | •  |            |    |            |    | 55 µg      | •  | 55 µg      | •  | 83 µg      | •  |
| Vitamin B12        |            |    |            |    |            |    |            |    | 2,5 µg     | ○  |            |    | 5 µg       | ○  |
| Folic Acid         |            |    | 800 µg     | •  |            |    |            |    | 400 µg     | •  | 200 µg     | ○  |            |    |
| L-Arginine         | 200 mg     | ○  | 250 mg     | ○  | 100 mg     | ○  |            |    |            |    | 30 mg      | ○  |            |    |
| L-Citrulline       |            |    |            |    | 800 mg     | ○  |            |    |            |    |            |    |            |    |

|                         |        |   |        |   |        |   |         |             |        |        |         |        |         |   |
|-------------------------|--------|---|--------|---|--------|---|---------|-------------|--------|--------|---------|--------|---------|---|
| $\alpha$ -Lipoic Acid   |        |   |        |   |        |   |         | 300 mg      | ○      |        |         | 800 mg | ●       |   |
| L-Carnitine             | 200 mg | ○ | 400 mg | ○ |        |   |         | 500 mg      | ○      | 30 mg  | ○       |        |         |   |
| N-Acetyl Cysteine (NAC) |        |   |        |   |        |   |         | 300 mg      | ○      | 600 mg | ●       |        |         |   |
| Coenzyme Q10            | 15 mg  | ○ | 15 mg  | ○ | 100 mg | ○ |         | 20 mg       | ○      |        |         | 200 mg | ●       |   |
| Astaxanthin             |        |   |        |   |        |   |         |             |        |        |         |        |         |   |
| D-Aspartic Acid (DAA)   |        |   |        |   | 80 mg  | ○ |         |             |        |        |         |        |         |   |
| Tribulus terrestris DE  |        |   |        |   |        |   | 300 mg  | ●           |        |        |         |        |         |   |
| Inositol                |        |   |        |   |        |   | 1000 mg | ●           | 100 mg | ○      | 1000 mg | ●      | 1000 mg | ● |
| $\alpha$ -Tocopherol    | 30 mg  | ● | 120 mg | ● | 30 mg  | ● |         |             |        |        | 30 mg   | ●      |         |   |
| Vitamin C               |        |   |        |   |        |   |         |             |        |        |         |        |         |   |
| DHA                     |        |   |        |   |        |   |         |             |        |        |         |        |         |   |
| Lycopene                |        |   |        |   |        |   |         | 4 mg        | ●      |        |         |        |         |   |
| Astragalus DE           |        |   |        |   |        |   |         |             |        |        |         |        |         |   |
| Damiana DE              |        |   |        |   |        |   |         |             |        |        |         |        |         |   |
| Nettle DE               |        |   |        |   |        |   |         |             |        |        |         |        |         |   |
| Catuba DE               | 50 mg  | □ |        |   |        |   |         |             |        |        |         |        |         |   |
| Ecklonia bicyclis DE    |        |   |        |   |        |   | 200 mg  | □           |        |        |         |        |         |   |
| L-Taurine               |        |   |        |   |        |   |         |             |        |        |         |        |         |   |
| Glutathione             |        |   | 80 mg  | □ |        |   |         |             |        |        |         |        |         |   |
| Glucosamine             |        |   |        |   |        |   | 150 mg  | □           |        |        |         |        |         |   |
| SOD                     |        |   |        |   |        |   |         |             |        |        |         |        |         |   |
| Vitamin D3              |        |   |        |   |        |   |         |             |        |        |         |        |         |   |
| Vitamin B1              |        |   |        |   |        |   |         | 1,1 mg      | □      |        |         |        |         |   |
| Vitamin B2              |        |   |        |   |        |   |         | 1,4 mg      | □      |        |         | 2,8 mg | □       |   |
| Vitamin B3              |        |   |        |   |        |   |         | 16 mg       | □      |        |         |        |         |   |
| Vitamin B5              |        |   |        |   |        |   |         | 6 mg        | □      |        |         |        |         |   |
| Vitamin B6              |        |   |        |   |        |   |         | 1,4 mg      | □      |        |         | 2,8 mg | □       |   |
| Biotin                  |        |   |        |   |        |   |         | 100 $\mu$ g | □      |        |         |        |         |   |
| Manganese               |        |   |        |   |        |   |         | 2 mg        | □      |        |         |        |         |   |

|  |  |  |  |  |   |  |  |
|--|--|--|--|--|---|--|--|
|  | DS 15<br> | DS 16<br> | DS 17<br> | DS 18<br> | DS 19<br> | DS 20<br> | DS 21<br> |
|--|--|--|--|--|---|--|--|

| Active ingredients      | S = 4,33   |     | S = 2,45   |     | S = 2,06   |     | S = 2,06   |     | S = 1      |     | S = 1,05   |     | S = 2      |     |
|-------------------------|------------|-----|------------|-----|------------|-----|------------|-----|------------|-----|------------|-----|------------|-----|
|                         | Daily dose | E V | Daily dose | E V | Daily dose | E V | Daily dose | E V | Daily dose | E V | Daily dose | E V | Daily dose | E V |
| Zinc                    | 15 mg      | ○   | 22,5 mg    | ○   | 10 mg      | ○   | 10 mg      | ○   | 6,5 mg     | ○   | 10 mg      | ○   |            |     |
| Selenium                | 50 µg      | ●   |            |     | 80 µg      | ●   | 50 µg      | ●   |            |     | 55 µg      | ●   |            |     |
| Vitamin B12             | 2,5 µg     | ○   |            |     |            |     | 1,5 µg     | ○   |            |     |            |     |            |     |
| Folic Acid              | 400 µg     | ●   | 300 µg     | ○   | 200 µg     | ○   | 200 µg     | ○   |            |     |            |     | 400 µg     | ●   |
| L-Arginine              |            |     | 2500 mg    | ●   |            |     | 200 mg     | ○   |            |     | 30 mg      | ○   |            |     |
| L-Citrulline            | 3000 mg    | ●   |            |     |            |     |            |     | 200 mg     | ○   |            |     |            |     |
| α-Lipoic Acid           |            |     |            |     |            |     |            |     |            |     |            |     |            |     |
| L-Carnitine             | 1000 mg    | ●   | 200 mg     | ○   |            |     | 400 mg     | ○   |            |     | 44,7 mg    | ○   |            |     |
| N-Acetyl Cysteine (NAC) |            |     |            |     |            |     |            |     |            |     |            |     |            |     |
| Coenzyme Q10            | 200 mg     | ●   |            |     |            |     | 100 mg     | ○   | 90 mg      | ○   |            |     |            |     |
| Astaxanthin             |            |     |            |     | 16 mg      | ●   | 10 mg      | ○   |            |     |            |     |            |     |
| D-Aspartic Acid (DAA)   |            |     |            |     |            |     |            |     | 1000 mg    | ○   |            |     |            |     |
| Tribulus terrestris DE  |            |     |            |     |            |     |            |     |            |     |            |     |            |     |
| Inositol                |            |     |            |     | 50 mg      | ○   |            |     |            |     | 500 mg     | ○   | 4000 mg    | ●   |
| α-Tocopherol            | 40 mg      | ●   | 30 mg      | ●   |            |     | 12 mg      | ○   |            |     |            |     |            |     |
| Vitamin C               | 80 mg      | ○   |            |     |            |     | 100 mg     | ○   |            |     | 180 mg     | ○   |            |     |
| DHA                     |            |     |            |     | 100 mg     | ○   |            |     |            |     |            |     |            |     |
| Lycopene                | 10 mg      | ●   |            |     |            |     |            |     |            |     |            |     |            |     |
| Astragalus DE           |            |     |            |     |            |     |            |     |            |     |            |     |            |     |
| Damiana DE              | 400 mg     | □   |            |     |            |     |            |     |            |     |            |     |            |     |
| Nettle DE               | 300 mg     | □   |            |     |            |     |            |     |            |     |            |     |            |     |
| Catuba DE               |            |     |            |     |            |     |            |     |            |     |            |     |            |     |
| Ecklonia bicyclis DE    |            |     |            |     |            |     |            |     |            |     |            |     |            |     |
| L-Taurine               |            |     |            |     |            |     |            |     |            |     | 300 mg     | □   |            |     |
| Glutathione             | 40 mg      | □   |            |     |            |     | 40 mg      | □   |            |     |            |     |            |     |
| Glucosamine             |            |     |            |     |            |     |            |     |            |     |            |     |            |     |
| SOD                     |            |     |            |     | 150 mg     | □   |            |     |            |     |            |     |            |     |

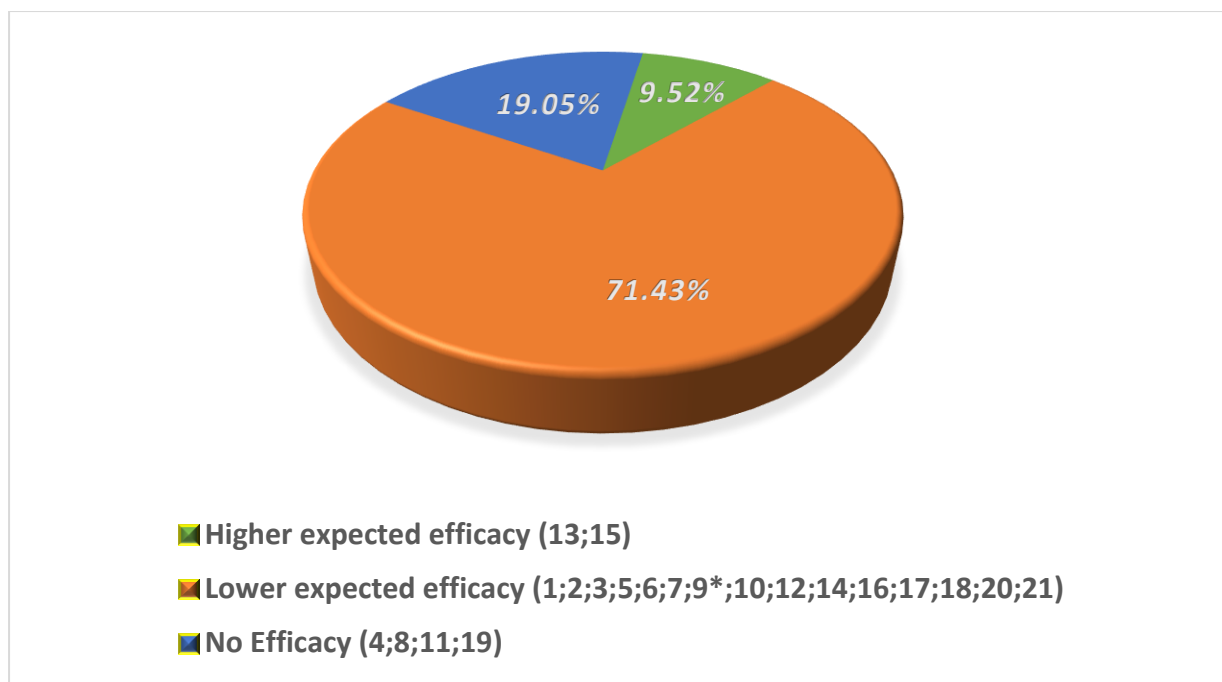
|            |  |  |  |  |  |  |                |   |  |  |  |  |  |  |
|------------|--|--|--|--|--|--|----------------|---|--|--|--|--|--|--|
| Vitamin D3 |  |  |  |  |  |  | 3,7<br>5<br>µg | □ |  |  |  |  |  |  |
| Vitamin B1 |  |  |  |  |  |  |                |   |  |  |  |  |  |  |
| Vitamin B2 |  |  |  |  |  |  |                |   |  |  |  |  |  |  |
| Vitamin B3 |  |  |  |  |  |  |                |   |  |  |  |  |  |  |
| Vitamin B5 |  |  |  |  |  |  |                |   |  |  |  |  |  |  |
| Vitamin B6 |  |  |  |  |  |  |                |   |  |  |  |  |  |  |
| Biotin     |  |  |  |  |  |  |                |   |  |  |  |  |  |  |
| Manganese  |  |  |  |  |  |  |                |   |  |  |  |  |  |  |

**Table 3.** List of dietary supplements (DS) with graphic representation of their expected efficacy (😊 higher, 😐 lower and 😞 no expected efficacy) and relative scores (S). For each supplement are indicated active ingredients, daily doses and their efficacy value (EV) represented by following symbols: (●) reported efficacy and achievement of mFED; (○) reported efficacy but below mFED; (□) unreported role in male fertility.

**DE:** Dry Extract; **SOD:** super oxide dismutase; **DHA:** docosahexaenoic acid.

Moreover, for each supplement, the scores of expected efficacy and the symbols summarizing the efficacy of their ingredients are reported. A detailed analysis of this table raised the following considerations: i) all supplements were mixtures of active ingredients; ii) in each supplement the number of ingredients ranged from 2 up to 17, with a mean number higher than 7; iii) 13 of 21 supplements contained at least 1 ingredient without proven efficacy; iv) 19 supplements had ingredients below mFED; v) indeed, 1 supplement contained even 7 ingredients dosed below mFED; vi) 1 supplement contained only active ingredients satisfying mFED; vii) The product number 9 had a nutrient reaching UL (zinc 40 mg/day); viii) zinc was the most used ingredient, followed by selenium, arginine, coenzyme Q, folic acid and carnitine. These substances were present in more than 50% of DS, whereas all the remaining ingredients were represented in 10% or less of products.

The distribution of DS into the 3 classes of efficacy is reported in Figure 2. Two DS out of 21 (9.5 %) were included in the higher expected efficacy group. The most part of remaining products (71.4 %) fell in the lower expected efficacy group, and four (19.1 %) in the group with no efficacy.



**Figure 2.** Distribution of supplements in classes of expected efficacy. \*This supplement has a content of Zinc reaching the UL.

## Discussion

This article aimed to evaluate the formulations of supplements for male infertility using the Italian market as sample. In general, there is still poor evidence in terms of large well-designed randomized and placebo-controlled trials availability, supporting the efficacy of nutraceutical products in the area of male reproductive health. Nevertheless, these products are commonly administered to infertile patients [8]. Since a medical prescription is not necessary to purchase dietary supplements, subjects seeking fertility may have an easy access to these products [10]. As a proof of concept, the Italian market of supplements generated 3.3 billion euros in 2019, with an increase of 4.3% compared to 2018 [13].

Whilst a rational use of supplements may be potentially beneficial for infertility, we need to stress that their uncontrolled use is potentially harmful for patients' health due to direct toxic effects and interaction with drugs or nutrients. In this respect, we were surprised to point out that all RCTs and metanalyses on zinc for male infertility relied on doses always exceeding the UL. Over this background, in the near future it would be desirable to better define thoughtful criteria for each supplement in use.

Our analysis found that beside the gap of literature, the market of food supplements is still supported by poor scientific evidence. The majority of DS contained a huge number of ingredients, up to 17. The mixture of such a high number of ingredients may generate different issues, including a low concentration of each substance (i.e. necessitating of two or more administrations to reach the daily effective dose), a large volume of pills and high risk of interactions. What is more, we found that some ingredients included in many DS had no scientific evidence of efficacy (egs. astragalus, vitamin D3, taurine and riboflavin). The formulation of pills with a large number of ingredients, some of which with uncertain benefits, denotes a gap of knowledge of potential biologic targets by manufacturers. Moreover, it has been reported that some plant extracts, present in many of these supplements, are likely to interact with drug metabolism [14,15]. This aspect raises further concerns on the safety of these products.

Very frequently, nutrients were present in DS at a dosage below mFED. This situation was more common among products with high number of ingredients. The administration of any active substance with dose below mFED appears as scientifically unjustified due to uncertainties in the therapeutic results. Differently, when the number of ingredients was small, the dose often satisfied mFED. Another major aspect in the evaluation of supplements concerns safety. Some ingredients, particularly when administrated in high doses, are not free from risks when used as dietary supplements. For example, folates can mask the B12 deficiency favoring the progression of neurological damage [16]. The combination of these two vitamins could have a synergic effect in improving homocysteine metabolism hence the sperm quality. Noteworthy that vitamin B12, when present, was rarely associated to folic acid [17,18]. Furthermore, zinc reduces the copper intestinal absorption interfering with its carrier [19]. In respect to this, we want to stress that one supplement on the market contained a dose of zinc reaching the UL.

On a positive note, our analysis revealed that some active ingredients with proven efficacy are frequently present in analyzed supplements. Previous studies demonstrated that some ingredients are particularly effective in specific patients' conditions. Substances with antioxidant properties are indicated in male accessory glands inflammation, both related to microbial and non-microbial origin. Several

studies performed in asthenozoospermic infertile patients, showed that the positive effect of selenium supplementation is dependent on the correct structure of mitochondrial capsule [20]. Carnitine supplementation induced a significant increase of sperm motility in cases of asthenozoospermia with preserved mitochondrial function [21]. Due to the key role of zinc in the processes of DNA compaction, administration of this micronutrient was successful in improving sperm morphology and DNA integrity in patients with prostate abnormalities [22].

Based on active ingredients reaching mFED we created a grading scale of supplements distinguishing three classes of expected efficacy. Three products were present in the higher class, some of which contained ineffective or underdosed ingredients. Most of the supplements fell in the lower group of expected efficacy. In this class, also a large number of ineffective or underdosed products was present. For an adequate evaluation of these classes, we considered the number of the effective ingredients as the most important criterion of efficacy. A relevant aspect was the use of ineffective or underdosed ingredients that should be absent or less than possible. Another parameter to evaluate a product was the presence a lower number of ingredients.

We acknowledge the application of a non-validated statistical method to calculate scores for each DS may represent a point of weakness of this study. However, as a point of strength, our scoring system relied on high quality evidence coming from RCTs or systematic review and meta-analyses of RCTs, which represents a reliable approach to critically weight the expected efficacy of dietary supplements. The same approach could be applied to evaluate products used in other clinically conditions.

In conclusion, this study showed that most DS on the Italian market contain ingredients with proven efficacy in male infertility. Nevertheless, a non-negligible number of DS are mixtures of substances with uncertain or unproven benefits, whose administration may be unhelpful or even harmful for infertile patients. On that basis, we believe manufacturers should carefully scrutiny scientific evidence before delivering each supplements' formulation. Accordingly, physicians should evaluate the composition of DS and the dose of each single constituent before considering their clinical use. Finally, the choice for DS should be tailored on to the specific patient's fertility problem.



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