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Review article

Omega-3 fatty acids dietary intake for oocyte quality in women undergoing assisted reproductive techniques: A systematic review

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ABSTRACT

Dietary intake of omega-3 polyunsaturated fats (PUFAs) may be associated with successful assisted reproductive techniques (ART). However, heterogeneous studies were conducted and opposing results were obtained. This systematic review aims to summarize the evidence on the effect of omega-3 dietary intake on oocyte and embryo quality for a positive ART outcome. The PRISMA 2020 statement was followed and the review protocol was registered with PROSPERO (CRD42021283881). Inclusion and exclusion criteria were: eligible studies examined women undergoing ART cycles whose diet was evaluated for omega-3 intake or experienced an increase in omega-3 compared with women who followed in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) but did not increase the omega-3 intake before the cycle. 5,412 records were identified and five studies were included in the analysis. Two studies focused on sub-fertile or infertile women specifically, yet all women in all studies went through IVF/ICSI procedures. All five studies demonstrated how omega-3 FAs may be beneficial by increasing the positive rate of ART outcomes and embryo quality evaluated according to morphology and morphokinetic parameters. More research focusing on comparable and/or equal outcomes is required to strengthen supporting evidence with the aim to provide valid recommendations for women seeking a pregnancy.

Introduction

Infertility is a multifactorial condition impacting up to 15% of reproductive-aged couples worldwide [21]. Large-scale studies estimate that 50% of factors affecting the individual spontaneous pregnancy are linked to female issues, 20–30% to male factors and 20–30% of events are due to both partners [5]. As a consequence, support through ART is often requested and more than 5 million children were born from ART interventions worldwide since its first application [44].

Female infertility includes multiple causes as ovarian dysfunction, endometriosis, tubes or uterus abnormalities, psychological and lifestyle factors [35]. For instance, age is a critical aspect as the biological decline of fertility naturally occurs at the age of 35 when the number and quality of eggs available deeply decrease. Consequently, pregnancy rates in older women are lower and miscarriage is likely to occur.

Lifestyle is an underestimated role-player. To maximise the success rate of the ART cycle, the general health and lifestyle of the couple are evaluated prior to treatment together with physical and BMI measures. Despite the close relation observed between obesity and poor reproductive outcome in the last 25 years, the mechanisms are still ambiguous [47]. Moreover, multiple studies highlighted the association between ovulation abnormalities and higher intakes of food categories such as dairy [1], animal proteins, carbohydrates, alcoholic beverages and caffeine [38]. The possible mechanisms impacting female fertility

Abbreviations: ALA, linolenic acid; AMH, anti-müllerian hormone; ART, assisted reproductive technology; ATP, adenosine triphosphate; βhCG, beta-human chorionic gonadotropin; BMI, body mass index; CI, confidence interval; CC2, second cell cycle; COH, controlled ovarian hyperstimulation; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; E_2, Estradiol levels; FA, fatty acids; FF, follicular fluid; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; MII, metaphase II; PUFAs, polyunsaturated fatty acids; TG, triacylglycerol; 2PN, 2 pronuclei.

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through dietary fats and omega-3 FA are still unclear. Fats are a heterogeneous group of substances that may be broadly divided into saturated (only single bonds) and unsaturated fats (presence of double or more bonds). All PUFAs and other FAs are carried in the circulation subdivided into triglycerides (3 FAs) within chylomicrons, cholesterol esters (2 FAs) and phospholipids (1 FA) within lipoproteins, and only in minimal part as free FAs to reach the target tissues, as the reproductive tract. Studies on bovines and humans show the correlation between plasma levels of free FAs and the follicular fluid that surrounds the developing oocyte, and DHA (22:6n-3) was found to be the most abundant omega-3 PUFA in this district. Studies on rodents and humans have demonstrated that a diet rich in omega-3 FAs or supplementation with omega-3 for at least 4 weeks increases the proportion of ALA, EPA (20:5n-3) and DHA in the follicle. Interestingly, in pathological conditions as benign ovarian tumors, the composition of omega-3 PUFAs is altered [46].

In adult mammals, FAs are typically stored in adipocytes as TGs to form a highly concentrated storage of metabolic energy. Among the other cell types of the organism, oocytes also accommodate high quantities of lipids in the form of droplets. The uptake of FAs in the eggs is precious since it represents the main source of ATP through mitochondrial oxidation. Although multiple biochemical aspects of oocyte development need further investigation, it is well-known that the FAs profile is important for oocyte development to the point where a negative energy balance may compromise fertility [27]. PUFAs seem to be involved in various pathways as far as fertility is concerned. The oocyte membrane composition is a key factor for fertilization since a successful conception requires a series of processes starting with the recognition and fusion of sperm and oocyte membranes [45]. Taking all the effects of omega-3 on female fertility into account, the aim of this work is to report the current evidence from human intervention and observational studies on the effect of omega-3 FAs on female fertility by focusing on the oocyte and embryo quality of women undergoing assisted reproductive techniques.

Methods

This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement [25,34]. A PROSPERO protocol was submitted and approved on December 3rd, 2021, with the registration number CRD42021283881.

Eligibility criteria

Inclusion and exclusion criteria were defined by following the PICOS framework (Table 1). Title and abstracts were examined to include studies on women experiencing ART cycles (P, "population") and following a dietary treatment or undergoing a dietary assessment that examines omega-3 FAs intake through food sources or supplements (I, "intervention") compared to women who followed assisted reproduction cycles and did not assume food with consistent intakes of omega-3 polyunsaturated fats or supplementation with omega-3 FAs (C, "comparison") to measure the impact on oocyte quality and ovarian reserve (O, "outcomes''). Finally, both observational and intervention studies were included (S, "study design"). In vitro and animal studies, reviews, conference abstracts, book chapters and other editorial material were not considered in this review. Interventions on pregnant or lactating women and studies considering the male population to assess fertility were excluded. Studies that examined the probability of natural conception in a given menstrual cycle without ART interventions were not considered. Studies in which the serum levels of omega-3 FAs or other PUFAs were measured as an outcome were excluded.

Information sources

A comprehensive search was conducted by one investigator via Pubmed, Embase, Scopus and Web of Science. Other relevant articles were included by considering the citation list of review articles. The last search was performed in November 2021.

Table 1
Population, intervention, comparator, outcome, and study design (PICOS) model of eligibility criteria.

Parameter	Inclusion criteria	Exclusion criteria
Population	Clinical and observational studies on women undergoing assisted reproduction techniques	In vitro experiments and animal studies, evaluation of male reproduction, pregnant and lactating women, general population with spontaneous pregnancies
Intervention	Intake of foods' source of omega-3 FA or supplementation with omega-3	Intervention with the Mediterranean diet, Western diet and other dietary patterns, intake of trans- fats and other fats
Comparator	Women undergoing assisted reproduction techniques who did not assume higher intakes of omega-3 fatty acids or supplementation with omega-3	
Outcomes	Oocyte and embryo quality, number of oocytes retrieved	Serum levels of omega-3
Study design	Experimental placebo-controlled studies	Review articles, book chapters, editorial material, dissertations and studies published in other languages than English before 2011

Abbreviations used: FA, fatty acids.

Search strategy

The search strategy throughout the four databases included the use of keywords by focusing on two different aspects of interest for this review: omega-3 polyunsaturated FAs and oocyte/embryo quality for assisted reproduction techniques. The following query and various combinations of terms were used: (("polyunsaturated fatty acids") OR pufa OR ("omega 3″) OR ("omega 3 fatty acids") OR ("docosahexaenoic acid") OR ("eicosapentaenoic acid") OR ("linolenic acid") OR ("alpha linolenic acid")) AND (fertility OR ivf OR ("in vitro fertilization") OR ("oocyte quality") OR ("good quality oocytes") OR ("oocytes number") OR ("oocytes retrieved") OR ("ovarian reserve") OR ("cumulus oocyte complex") OR ("meiotic spindle") OR ("oocyte competence") OR ("oocyte maturation") OR ("oocyte activation") OR oestradiol OR ("embryo morphology") OR ("high pregnancy")). Filters were applied to focus on human studies, English as the only language and publication date within the last 10 years (from 2011). Restrictions were adopted regarding the study type to exclusively consider clinical studies, clinical trials, major clinical studies, observational studies, prospective cohort studies, retrospective case-control and randomized controlled trials.

Selection process

A single reviewer independently screened and evaluated eligibility to include studies in the report. Articles were first screened by DOI to exclude duplicates. Unique articles were then assessed for eligibility and included in the systematic review if they met the following criteria: (i) observational study or randomized controlled trial; (ii) population limited to women undergoing ART cycles; (iii) intake of omega-3 rich food or supplied with omega-3 fatty acids; (iv) parameters related to embryo/oocyte quality and number as outcomes of interest. First, articles were screened by title and abstract. Afterwards, possible relevant articles were read meticulously to evaluate eligibility. No automation tools were adopted in the process.

Study risk of bias and certainty assessment

The evaluation of the risk of bias in randomized intervention studies included in the review was done by a single reviewer through RoB 2.0 tool [40]. The grading of Recommendations Assessment, Development and Evaluation (GRADE) system was adopted to evaluate the certainty of evidence.

Results

Study selection

Literature extraction and selection are described in the flow chart created in accordance with PRISMA guidelines (Fig. 1). The searches retrieved 5,412 results among which 1,920 articles were identified as duplicates and removed. 3,298 articles were screened for relevance: from these, 3,285 articles were excluded for the following reasons: irrelevant title and abstract (n = 1,728), animal or in vitro studies, reviews and articles published in other languages than English and earlier than 2011, studies evaluating male fertility or pregnant and lactating women (n = 1,557). A total of 13 reports were read and assessed for eligibility and discarded in case they were referring to women conceiving naturally (n = 1), none or inaccurate dietary intervention or assessment was performed (n = 4) or described the study design and protocol of an included study (n = 3). As a result, a total of 5 studies were included in the systematic review.

Study characteristics

The fundamental characteristics of each study are outlined in Table 2. Three studies were conducted in Europe [15,22,33] and two in the Middle East [2,17]. Three identified studies are RCTs. Among them, two are double-blinded [2,22] and one is open-label and defined as a prospective pilot study [33]. Two retrieved studies are longitudinal studies [15,17]. All women enrolled received ART treatment: ICSI [2] specifically or either IVF or ICSI treatments within the same study [15,17,22,33]. Eight women [33] had to interrupt IVF before oocyte retrieval due to ovarian hyperstimulation syndrome and intention-to-treat analysis was adopted. The other studies did not report any deviation from the protocol.

Participants' characteristics and intervention

Al-Alousi et al. [2] included 20 sub-fertile women with a BMI in the normal, overweight and obese range (BMI > 18, < 35 kg/m^2) excluding cases of medical disorders, consumption of any medications that could impact the hormonal assay, women that were on a diet in the previous three months, smokers and alcohol consumers, or whose who took omega-3 PUFAs supplements in the previous three months. They randomly treated the first group with omega-3 (one daily capsule composed of 180 g EPA and 120 g DHA) while the second group was given a placebo (500 g paraffin daily) before starting the ICSI protocol. The study was double-blinded.

Nouri et al. [33] ran a pilot study with 100 women of age between 19 and 42 years and BMI higher than 20 and lower than 28 kg/m^2. Subjects taking vitamins or multi-nutrients, other than folic acid alone for the

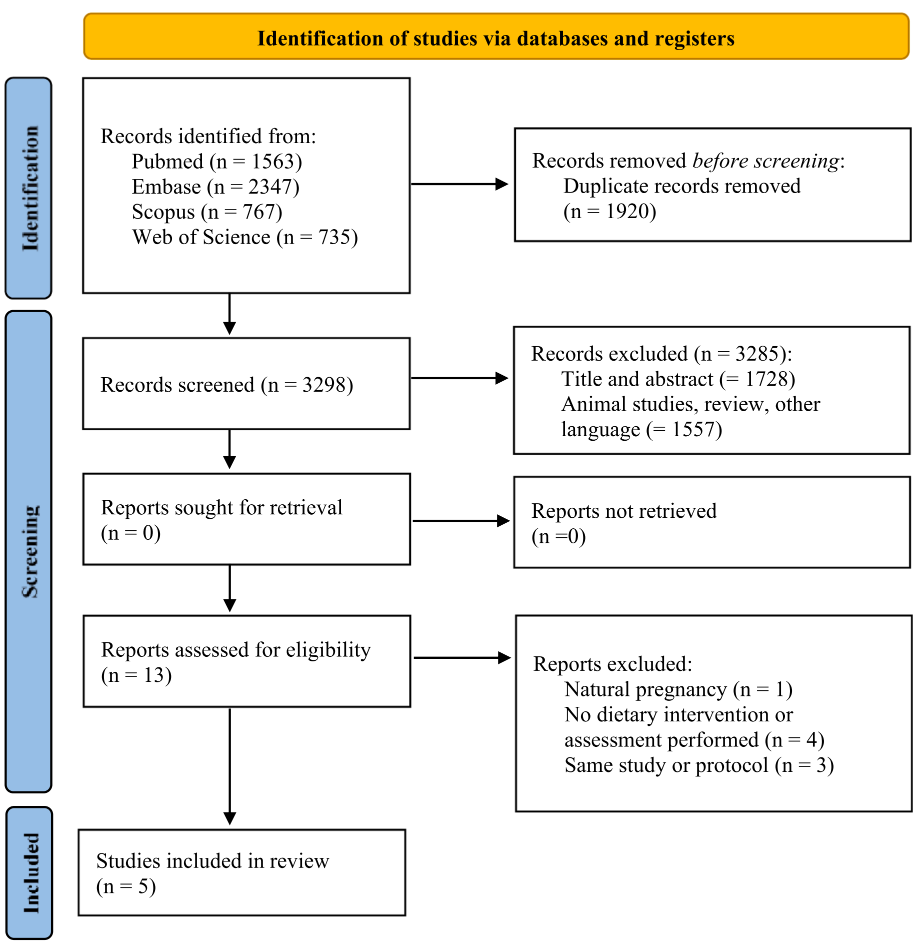

Fig. 1. Flow diagram of the study selection process according to PRISMA 2020 guidelines.

Table 2
Characteristics of the eligible studies.

Ref.	[33]	[2]	[22]	[15]	[17]
Location	Austria	Iraq	United Kingdom	Netherlands	Iran
Study Design	Open-label pilot study, randomised controlled trial	Single-blind randomised, controlled trial	Double-blind, randomised, controlled trial	Prospective cohort study	Descriptive longitudinal study
N. of participants	100	120	102	235	174
Inclusion criteria	Women with BMI > 20, < 28 kg/m2, 19–42 years, undergoing IVF	Subfertile women, BMI > 18, < 35 kg/m2, 20–40 years, undergoing IVF, and fresh sperm sample (not aspirated)	Women with BMI ≤ 20, ≥32 kg/m2, 18–41 years, undergoing IVF/ICSI	Women undergoing IVF/ICSI treatment	Infertile women undergoing IVF/ICSI
Exclusion criteria	Women assuming vitamins or multi-nutrients, other than folic acid alone for the previous 3 months	Women who have medical disorders, artificial sperm collection use of any medications in the last 12 weeks that may influence hormonal assay, history of any diet in the previous three months, tobacco and alcohol consumption, and supplementation of n-3 PUFAs in the past three months	Medical contraindication to ART treatment or to a specific dietary intervention, previously diagnosed diabetes, taking prescribed medication or herbal remedies apart from simple painkillers, and eating oily fish (as defined by the UK Food Standards Agency) more than once a week	Women experiencing conditions that impair IVF outcome	Change on the diet or following special diet, metabolic disorders, assuming drugs affecting the metabolism, anatomic abnormalities or surgery in the uterus or tubes, using a surrogate, assuming alcohol, smokers
Exposure	One tablet a day containing	One tablet a day containing 1000 mg omega-3 composed of 180 mg EPA and 120 mg DHA	Dietary intervention with olive oil for cooking, an olive oil-based spread, and a daily supplement drink enriched with Vitamin D (10 μg daily) and marine omega-3 FA (2 g daily)	Validated FFQ to evaluate long-chain PUFAs intake	Validated FFQ including 168 food items
Control	One capsule containing 400 μg folic acid	Capsule containing 500 mg paraffin	Sunflower seed oil for cooking, a sunflower seed oil–based spread, and a daily supplement drink without EPA, DHA, or vitamin D	–	–
Duration	30–39 days	8 weeks	6 weeks	Assessment of nutritional intake over 4 weeks	Assessment of nutritional intake over 1 year
Fertility Parameters Examined	Embryo quality and pregnancy rates	Embryo grading and ICSI outcome parameters	Mean period in hours for CC2	Embryo quality, number of follicles and estradiol levels	MII oocytes, fertilization rate, embryo quality, biochemical and clinical pregnancy
Results	Higher embryo quality in the study group. No significant difference in pregnancy rates.	Higher 2PN zygote, fertilization rate, cleavage rate, grade 1, and endometrial thickness. Higher pregnancy rate but with no significant difference.	Overall improvement in the morphokinetic markers of embryo quality	The number of follicles and E2 response was inversely associated with high intakes of total omega-3. Significant positive associations between embryo morphology and total omega-3 intake and DHA. High omega-6-to-omega-3 ratio was positively associated with the number of follicles.	Significant increase in MII oocytes in the higher tertile of ALA; lower MII oocytes in the higher tertile of EPA and DHA; higher fertilization rate with higher intake of ALA and EPA.
Conclusion	A multivitamin supplementation that includes omega-3 FA is beneficial in terms of fertilization rates and embryo quality	A short period of dietary supplementation alters the rate of embryo cleavage by increasing the efficiency of embryo development before the implant	A high intake of omega-3-rich oil led to a significant increase in ovulation and follicles development	Dietary long-chain PUFAs significantly contribute to E2 levels, number of follicles and embryo morphology	ALA is directly associated with the number of oocytes. A high intake of EPA and DHA is associated with fewer MII oocytes. PUFAs could improve embryo quality.
Risk of Bias	High	Some concerns	Low	NA	NA
Study Quality	Very low	Very low	Moderate	Low	Low

Abbreviations used: ALA, linolenic acid; ART, assisted reproductive technology; BMI, body mass index; CC2, second cell cycle; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; E_2, Estradiol levels; FA, fatty acids; FFQ, food frequency questionnaire; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; PUFAs, polyunsaturated fatty acids; MII, metaphase II; 2PN, 2 pronuclei.

previous 3 months, were excluded. The randomization was based on the day of the first visit to the IVF clinic and according to this principle, women were given the treatment (n = 50) that consisted of (i) one tablet containing 800 µg folic acid, 70 µg selenium, 30 mg vitamin E, 4 mg catechins, 12 mg glycyrrhizin, 32 mg diosgenin and 90 mg damiana; (ii) one soft capsule containing 500 mg omega-3-fatty acids. The control group was given a pill containing 400 µg folic acid only. The study was unblinded so all subjects were aware of whether they were part of the study or the control group. All women had to carry out the treatment for 28 days up to a maximum of 56 days so no specific treatment period was adopted.

Kermack et al. [22] enrolled 102 couples in the study if females met the following inclusion criteria: age between 18 and 41, BMI > 18, < 32 kg/m^2 and use of partner's sperm to conceive. Exclusion criteria were established as follows: more than two previous unsuccessful IVF cycles, low ovarian reserve according to the AMH level, any medical contraindication to IVF or IVF-ICSI treatment or to the specific dietary intervention, previously diagnosed diabetes, the use of prescribed medication or herbal remedies other than simple analgesia, or eating fatty fish more than once a week. The intervention for the study group consisted of a 6-weeks dietary intervention with olive oil for cooking, an olive oil-based spread, a daily supplement drink enriched with Vitamin D (10 µg daily) and marine omega-3 FAs (2 g daily). The control group received sunflower seed oil for cooking, a sunflower seed oil-based spread, and a daily supplement drink without EPA, DHA, or vitamin D. Consumed drinks were presented as a white label to the participants to ensure the study blindness.

The 235 women who participated in the study conducted by Hammiche et al. [15] were undergoing IVF/ICSI treatment. In case of oocyte donation, endometrioma, hydrosalpinx or other conditions that could impair the IVF outcome, subjects were excluded from the study.

In their study, Jahangirifar and colleagues [17] enrolled 217 infertile women who were facing unexplained infertility or ovarian failure, with no significant change in diet or following a special diet within the previous 3 months, without metabolic diseases or using drugs affecting the metabolism, without anatomic abnormalities, endometriosis and surgery in the uterus and tubes, not using alcoholic beverages or smoking. They measured multiple parameters associated with the ART outcome in the subjects grouped in tertiles according to the consumption of fatty acids. In both longitudinal studies [15,17], the dietary assessment was performed by submitting a validated food frequency questionnaire. Hammiche et al. collected the general characteristics and nutritional intake over the previous 4 weeks before the day of COH and calculated the total omega-3 intake as the sum of ALA, EPA, and DHA [15]. Instead, Jahangirifar et al. evaluated the food consumption of the participants over the previous year, calculated the number of food items for one day per person and evaluated the quantity of different types of fatty acids, including ALA, EPA and DHA.

Exposure and outcomes

Al-Alousi et al. [2] assessed embryo quality based on morphological grading as the primary endpoint and evaluated the difference in multiple ICSI outcome parameters as the number of follicles, rate of the follicle to oocyte retrieval (fertilization rate), the ratio of number of follicles related to the number of injected oocytes. Pregnancy rates were evaluated and also associated with the ovarian stimulation protocol to exclude any influence of this practice. On day 3 after oocyte retrieval, Nouri et al. [33] measured the embryo quality according to the number of cells and fragmentation rate, by differentiating good quality embryos (at least 6 cells and a fragmentation rate of less than 20%) and poor quality ones (less than 6 cells and a fragmentation rate higher or equal to 20%). Women that had at least one embryo of good quality were separated from those without any. The secondary outcome was the clinical pregnancy defined as an intact pregnancy with a positive heartbeat on vaginal ultrasound. Kermack et al. [22] measured a series of morphokinetic parameters of healthy embryo development as a primary endpoint. Multiple secondary outcomes were considered, such as blood measurements of EPA, DHA, and vitamin D, additional validated parameters of embryo development associated with an increased chance of development to a blastocyst, implantation, clinical pregnancy, pregnancy rate and day 3 and day 5 KIDScores. The primary outcome of the study conducted by Hammiche et al. [15] was embryo morphology which was presented as the average score of embryo quality (1 for perfect embryos, 2 for good ones) of all embryos retrieved from each woman. Estradiol levels (E_2) and the number of follicles obtained in a single IVF cycle were also recorded. The laboratory measurements reported by Jahangirifar et al. [17] included the average number of total oocytes and MII oocytes, fertilization rate, the ratio of good and poor quality embryos and biochemical and clinical pregnancy, defined as the presence of βhCG in serum 12 days after embryo transfer and detection of one or more gestational sacs during transvaginal scan 3 weeks after embryo transfer, respectively. The quality of the embryos was assessed with a four-point score on day 3 according to their cleavage, absence of fragmentation, absence of irregularities in blastomere size or shape and 8-cell stage on day 3.

Risk of bias in studies

The main remarks of the analysis are considered herein. One study followed an intention-to-treat approach by clearly stating so in the methods [33]. The remaining two studies followed a per-protocol approach [2,22]. With regard to the risk of bias from the randomization process: precise information on the adoption of a computer-based allocation sequence of randomization was not specified in any study; one study used permuted block randomization with blocks of varying size and allocation concealment [22]; Nouri et al. [33] randomized the intervention according to the first visit of the treated women; only one study indicated that randomization was carried out without providing further details [2]). A double-blind study protocol was adopted and clearly stated only in one RCT [22]; Al-Alousi et al. [2] treated the subjects with omega-3 and control pills that had the same appearance, therefore blindness was applied but no indication on researchers blindness is provided. No missing data was reported in any study and trials were analysed according to a pre-specified plan in all cases. The

Table 3
Risk of bias analysis of per-protocol interventions.

REF	Randomisation process	Deviations from the intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	**Overall**
[16]	+	+	+	+	+	+
[17]	!	+	+	+	+	!

outcome of the analysis is graphically reported in Table 3 and Table 4.

Results of individual studies

None of the studies reported significant differences in the basic characteristics of participants between the study and control groups. Nouri et al. [33] only focused on embryo quality and pregnancy rates. The chance to get at least one embryo of good quality was higher in the study group compared to the placebo (58.0% chance against 36.0%, p = 0.045, relative risk = 1.611, IC 1.009–2.597). However, no significant difference was observed in the two groups regarding pregnancy rates (40.0% rate in the study group against 26.0% in the placebo group, p = 0.141). Al Alousi et al. [2] measured in detail the multiple parameters that describe ICSI outcomes. Among them, a significant difference between study and control groups was evaluated for the number of 2PN zygote (5.13 ± 0.44 vs 3.60 ± 0.46, p < 0.01), fertilization rate (67.89 ± 3.60 vs 51.92 ± 4.20, p < 0.005), cleavage rate (6.11 ± 0.47 vs 5.41 ± 0.49, p < 0.001), grade 1(2.16 ± 0.28 vs 0.67 ± 0.13, p < 0.001), and endometrial thickness (11.37 mm ± 0.12 vs 10.87 mm ± 0.21, p < 0.04). No significant difference in pregnancy rates was measured. Kermack et al. [22] analysed 356 embryos in the study group and 394 in the control group. Statistically significant reductions in CC4 and S3 times (reduction of 0.45 h with P < 0.001 and reduction of 0.23 h with P = 0.02, respectively) are highlighted in the study, together with an increase in KIDScore on day 3 (0.18 increase, P = 0.05). No other morphokinetic parameter was statistically different in the group that consumed a diet rich in omega-3 FAs in comparison to the control group. Hammiche et al. [15] used the β adjusted regression coefficient to express results according to the relative effect per gram of LC-PUFA intake. The authors reported the association between embryo morphology and total omega-3 intake (β = 0.16 (0.08), P ≤ 0.05), ALA (β = 0.56 (0.26), P ≤ 0.05) and DHA (β = 0.18 (0.09), P ≤ 0.05). Baseline E2 was positively associated with high intakes of ALA (β = 89.3 (36.7), P ≤ 0.05) and estradiol response was negatively associated with high intakes of EPA (β = -1100.2 (498.4), P ≤ 0.05) and DHA (β = -1065 (492.6), P ≤ 0.05). The number of follicles was inversely associated with high intakes of total omega-3 (β = -1.79 (0.58), P ≤ 0.01), EPA (β = -1.49 (0.49), P ≤ 0.01), and DHA (β = -1.60 (0.49), P ≤ 0.01). Taking the scope of this review into account, Jahangirifar et al. [17] determined the association between tertiles of ALA, EPA and DHA intakes and fertility markers. A significant increase in the number of retrieved MII oocytes in the group of women that consumed higher intakes of ALA (8.69 ± 1 compared with 7.87 ± 1.1 of the lower tertile, p = 0.005 adjusted for cofounders), lower MII oocytes in the higher tertile of EPA (4.70 ± 3.4 vs 8.57 ± 0.6 of T_2, p = 0.001, adjusted for cofounders) and DHA (7.36 ± 0.4 vs 8.5 ± 0.3 of T_2, p = 0.001, adjusted for cofounders) and a higher fertilization rate with higher intake of ALA (0.70 ± 0.05 of T_3 vs 0.64 ± 0.05 of T_1, p = 0.007, adjusted for cofounders) and EPA (0.71 ± 0.1 of T_3 vs 0.68 ± 0.03 of T_1, p = 0.003, adjusted for cofounders).

Table 4
Risk of bias analysis of intention-to-treat interventions.

REF	Randomisation process	Deviations from the intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	**Overall**
[14]	-	-	+	-	+	-

Certainty of evidence

The rating process based on the confidence of evidence is synthesized in Table 5.

Whenever the risk of bias was rated low, the score was not reduced; 'some concerns' RoB outcome was associated with a serious risk; 'high' was associated with 'very serious' ranking. Inconsistency is evaluated from the similarity of point estimates, the extent of overlap of confidence intervals, and statistical criteria including tests of heterogeneity and I^2. Heterogeneity was assessed in one study [29], with Levene's Test for equality of variances. The intervention that was implemented by Nouri et al. [33] consisted of a daily intake of a capsule rich in micronutrients and antioxidants that were studied for the positive effect on fertility. In addition, only one soft capsule of omega-3 was given to the women included in the study. Although the omega-3 intake was explicit, the intake of all the other components cannot guarantee that the results are caused by omega-3 only. The intervention and outcome of the other two studies are defined as direct [2,22].

In this review, the test for heterogeneity and I^2 were not performed as no metanalysis was conducted. Therefore, the quality assessment relies on the other four principles.

Imprecision is measured upon the difference between intervention and control for each outcome, by focusing on the 95% CI. In two studies only [22,33], the reported results showed significant CIs. Publication bias reported as "negative studies" are more likely to be discarded and "positive studies" are published with higher frequency. Moreover, when reviews are related to a rising aspect of research, only a few initial studies are available. Consequently, effects are overestimated while "negative" studies often experience a delay in publication. "Negative studies" may even be rejected or get delayed at different stages (e.g. preliminary and pilot studies, journal selection, peer review). This review is mainly based on small RCTs and one observational study is included. Therefore, the publication bias is high. As a result of the assessment, only one [22] out of three RCTs has been evaluated as a moderate-quality study. Therefore, possibly the true effect of a dietary intake of omega-3 FA on fertility is substantially different from the actual measure. Two studies were rated as very low quality [2,33] mainly due to the risk of bias analysis. The quality of the longitudinal studies [15,17] was rated as low and rating up was not applicable.

Discussion

In this systematic review, the relationship between fertility and nutrition has been evaluated by focusing on the emerging association between omega-3 intake through diet and oocyte quality.

Biological Plausibility

The interest was raised by the recent and promising findings of both animal and human studies. As a first step, multiple animal studies were conducted to test the biological plausibility of the relation between omega-3 intake and fertility focusing on their action on key processes such as steroidogenesis, oocyte maturation and embryo implantation but also male potency and sperm quality [7,32,37,39,41]. As a recent remark, alterations in the murine ovarian gene expression after a high-fat diet was improved upon supplementation with omega-3 FAs [16].

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Table 5
Certainty of evidence.

Ref.	Study design	Initial quality	Risk of Bias	Inconsistency	Indirectness	Imprecision	Publication bias	Assessed quality
[33]	RCT	High	Very serious	NA	Serious	NA	Serious	**Very low**
[2]	RCT	High	Serious	NA	NA	Serious	Serious	**Very low**
[22]	RCT	High	NA	NA	NA	NA	Serious	**Moderate**
[15]	Prospective study	Low	NA	NA	NA	NA	NA	**Low**
[17]	Longitudinal study	Low	NA	NA	NA	NA	NA	**Low**

Abbreviations used: RCT, randomized controlled trial.

Omega-3 intake and human female reproduction

Taking this noticeable evidence into account, studies on humans have been recently conducted to understand if the association between omega-3 and enrichment in women's reproductive capability was significant as well. The effect on key aspects of female reproduction was investigated leading to conflicting results, with positive, negative or no association retrieved [3,9,43]. Among the positive effects, women with lower omega-3 PUFAs intake had reduced fecundability compared to women in the other quartiles [42], estradiol levels were higher in women who consumed more ALA [15] and DHA was associated with reduced risk of anovulation and increased estradiol [31]. Observational and, more recently, intervention studies have focused on women undergoing IVF/ICSI procedures to deeply analyse oocyte and embryo mechanisms before implantation, together with pregnancy rates. Similarly, the results are in disagreement [11,15,19,20,22,28,29,36]. The conditions in which omega-3 effects were evaluated are heterogeneous and the measured outcomes differ consistently.

In this review, we decided to summarize the evidence reported in the literature on omega-3 and reproduction by specifically focusing on the possible effect of omega-3 dietary intake on oocyte and embryo quality during assisted reproductive technology procedures rather than natural conception. The guidelines reported in the most recent version of the PRISMA Statement were followed meticulously. Specifically, the risk of bias was evaluated with the RoB tool but no discussion occurred among multiple reviewers to report a conveyed assessment. Therefore, bias might have been introduced. As a first constraint, the number of participants across the studies of this review is a matter of concern. In comparison with other trials conducted to investigate similar matters [10], the number of women included is limited.

Strengths and limitations

One of the major limitations is represented by the poor homogeneity of the outcome measures. Nouri et al. [33] ran an unblinded study focused on the measurement of fertilization rates (a parameter that evaluates ICSI success) and embryo quality, based on the number of cells and fragmentation rate. Embryo morphology according to GRADE criteria was followed according to cells size and fragmentation [2] of the embryo morphology as an outcome based on either the number of blastomeres or the developmental stage [15], or embryo cleavage, fragmentation and irregularities in size or shape [17]. Therefore, multiple definitions of good embryo morphology were embraced. The measure of the embryo quality was reorganized into the evaluation of morphokinetic parameters of the embryo in the study by Kermack et al. [22]. While embryo morphology was found to be a poor predictor of embryo development and pregnancy rate, morphokinetic evaluations are based on the accuracy granted by the knowledge of precise kinetic of development before each embryo transfer to reduce invasiveness and predict embryo competence in advance [8]. Therefore, morphokinetic parameters represent powerful markers of choice to assess embryo quality. On a whole, the results summarized in our review represent primitive but encouraging evidence of the possible beneficial effect of omega-3 increased intake through diet to favour IVF/ICSI outcomes. Moreover, omega-3 FAs introduced through a multivitamin or single-compound supplementation may be beneficial in terms of fertilization rates and embryo quality [2,33], as well as a high intake of oils rich in omega-3 was observed to improve E2 levels, ovulation, follicles development and embryo morphology [15,22]. However, additional studies have to be conducted to improve the understanding of omega-3 and fertility parameters, as higher intakes of EPA and DHA might decrease the number of MII oocytes and E2 response [15,17], probably due to a decline in prostaglandin F2α that positively contributes to the follicle growth and ovulation.

Principal findings

Although it goes beyond the scope of our research, to provide a broader understanding of the relationship between omega-3 and female fecundity it is necessary to report that multiple studies focused on the association between a higher daily intake of omega-3 PUFAs (via supplements or food sources) and natural pregnancy rates in women of reproductive age [3,4,31,43].

The iFish Study published in April 2021 [12] is briefly described as an example of an investigation on the effect of fish consumption on PUFA concentrations and markers of inflammation and oxidative stress in women of childbearing age. Results suggest that the consumption of two portions of fish per week may reinforce serum concentrations of EPA, DHA and total n-3 PUFAs, and a lower n-6:n-3 ratio compared to those in the no fish or 1 portion per week group (all $p < 0.05$). This conclusion confirms that omega-3 PUFAs are biomarkers of usual dietary intake as reported by Baylin et al. [6]. However, no effects on oxidative stress and inflammation were observed during the trial.

The analysis of the influence of serum omega-3 PUFAs concentration on reproductive functions has been an active area of research. Studies with this scope lead to conflicting results [13,18,19,20,24,30]. Interestingly, even though increased dietary intakes of PUFAs have a positive influence on female fertility, whenever their concentration becomes too high it might have a negative impact on important reproductive mechanisms such as oocyte quality, endometrial receptivity and subsequent embryo implantation rates [13,20]. This suggests how the measurement of serum omega-3 PUFAs levels has not always produced clear results and more heterogeneous studies have to be directed. The mentioned trend applies not only to serum concentrations of FAs but also to their content within the FF. Kermack et al. [23] observed a higher concentration of EPA and DHA in the follicular fluid of women that received the 6-weeks dietary treatment with omega-3 FAs and vitamin D was significantly higher and linked to pregnancy success. However, in multiple studies, a positive association was found between serum omega-3 PUFAs and fertility parameters. Chiu et al. [11] measured serum FAs of 100 women from the EARTH cohort to calculate the probability of implantation, clinical pregnancy and live birth per cycle. They reported that higher levels of serum omega-3 PUFAs were positively associated with pregnancy and live birth rate; furthermore, ratios of omega-6: omega-3 were inversely associated with peak estradiol levels that are markers of ovarian response to stimulation.

The beneficial role of omega-3 PUFAs was confirmed in a recent case-control study that analysed the fatty acid profile of 922 oocytes from obese and normal-weight women undergoing IVF. The composition in FA differs in oocytes of obese or overweight women. More specifically, lower levels of omega-3 PUFAs FA and the lowest omega-6:omega-3 ratios were recorded in obese women who also had higher DHA levels than overweight and normal-weight women [26].

Future directions

On the whole, the effects of omega-3 PUFAs should include a complete assessment of their dietary intake, serum concentration and follicular fluid composition. The literature fails to elaborate any strong and unbiased evidence, however, the further research should be encouraged to pave the way for future intervention. In particular large studies are needed to better understand the role of omega-3 PUFAs contextualized in the overall dietary habits and adoption of a healthy lifestyle by both partners.

Results seem encouraging in females and their male counterparts [14]. Still, the available evidence is not straightforward enough and more research is required to determine the extent of the effect of omega-3 PUFAs in women undergoing assisted reproductive techniques.

Conclusions

The scope of this systematic review was to investigate the current evidence on the effect of a dietary intervention that includes omega-3 FAs or a dietary assessment focused on their intakes among women undergoing assisted reproductive technology procedures to improve oocyte and embryo quality. While only a few studies were found to be eligible, yet the positive impact of omega-3 on key parameters for female fertility is encouraging and supported by biologic plausibility. Despite the heterogeneity, the emerging results pave the way for future intervention studies to strengthen the evidence on the relationship between nutrition and fertility.

Key Message

This systematic review is focused on the studies exploring assisted reproductive technology conditions rather than natural conception. The relationship between fertility and nutrition has been evaluated by focusing on the association between omega-3 intake through diet and oocyte quality. The positive effects need to be contextualized in the overall dietary habits and adoption of a healthy lifestyle by both partners.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejogrb.2022.06.019.

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