Effect of a 6-week "Mediterranean" dietary intervention on in vitro human embryo development: the Preconception Dietary Supplements in Assisted Reproduction double-blinded randomized controlled trial

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Objective: To study the impact of increased dietary intake of omega-3 fatty acids, vitamin D, and olive oil for 6 weeks before in vitro fertilization (IVF) or IVF–intracytoplasmic sperm injection (ICSI) on morphokinetic markers of early embryo development.
Design: A double-blinded randomized controlled trial.
Setting: Academic IVF unit.
Patient(s): A total of 111 couples undergoing IVF or IVF-ICSI were recruited.
Interventions(s): Fifty-five couples received the 6-week study intervention of a daily supplement drink enriched with omega-3 fatty acids and vitamin D plus additional olive oil and olive oil–based spread, and 56 couples received the control intervention.
Main Outcome Measure(s): The primary end point for the study was the time taken for completion of the second cell cycle after fertilization (CC2). Secondary end points included time to complete the third and fourth cell cycles (CC3 and CC4), the synchrony of the second and third cell cycles (S2 and S3), and the day 3 and day 5 Known Implantation Data Scores (KIDScores).
Result(s): There was no difference in CC2 between the two groups. However, CC4 was accelerated in the study group compared with the control group, and a significantly shortened S3 as well as an increase in KIDScore on day 3 were observed, indicating improved embryo quality in the study group.
Conclusion(s): This study demonstrates that a short period of dietary supplementation alters the rate of embryo cleavage. Further research is required to investigate the mechanisms that regulate this effect, and whether the impact on embryo development translates into improved clinical outcomes.

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A growing body of evidence from prospective cohort observational studies indicates that the periconceptional nutritional status of both father and mother affects early fetal development and perinatal and long-term health of the offspring (1). In recent years, a number of observational studies have indicated that variations in preconceptional diet may affect early embryo development. Particular interest has been afforded to the possible effects of a Mediterranean diet (2, 3). An observational study demonstrated an increased chance that embryos would form a blastocyst when they were from women who reported consuming higher quantities of fruit and fish and a decreased chance of blastocyst formation in those consuming more red meat or who were on a weight loss diet (4). Furthermore, a prospective observational study reported that a Mediterranean diet high in vegetable oils, fish, vegetables, and legumes and low in carbohydrate-rich snacks was positively associated with red blood cell folate and vitamin B6 in blood and follicular fluid and with a 40% reported increase in the probability of achieving a pregnancy (5). A more recent cohort study demonstrated a significantly increased clinical pregnancy rate and almost double the live birth rate in couples consuming a more Mediterranean diet (according to the validated MedDietScore) compared with those who did not (6). The Prevención con Dieta Mediterránea (PREDIMED) study (7) investigated the effect of the Mediterranean diet on cardiovascular disease, comparing specific dietary advice and nuts or extra-virgin olive oil (intervention groups) versus guidance on decreasing fat intake (control group). A significant difference in mortality between those adhering to the Mediterranean diet and those in the low-fat control group was demonstrated; however, that trial has recently been criticized owing to the complexities of ensuring that a specified diet is followed in a randomized trial (8).

Acknowledging the difficulties of significantly altering overall diet in a study setting, the Preconception Dietary Supplements in Assisted Reproduction (PREPARE) trial was developed to examine the importance of key components of the Mediterranean diet, including olive oil, omega-3 fatty acids (FAs) from seafood, and vitamin D. The effect of increased dietary intake of the omega-3 FAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on markers of embryo morphology is unclear, with some studies demonstrating a benefit (9) and others showing no advantage (10). It is recognized that FAs are required by the developing embryo as a source of energy (11) and for synthesis of newly forming cell membranes (12). There is also some evidence that increased levels of omega-3 FAs in the cell membrane may increase the gap junction capacity of the morula (13).

The importance of vitamin D as a determinant of embryo development is unclear, although it has also been implicated as a key factor in fertilization, affecting sperm-egg binding and the activity of acrosine which digests the zona pellucida (14). However, despite evidence from a recent prospective cross-sectional study demonstrating a significantly decreased pregnancy rate in women with a serum level <20 ng/mL (15) and the inclusion of vitamin D in many conception multivitamin preparations, increased levels of vitamin D in follicular fluid have been associated with poorer oocyte (16) and embryo (17) quality.

Although a growing body of literature suggests that preconceptional exposure to key components of the Mediterranean diet may affect gamete and embryo development, the reported data are largely observational and there remains a lack of intervention studies designed to clarify the validity of this association. The PREPARE trial (18) was a prospective double-blinded randomized controlled trial investigating whether a drink high in omega-3 FAs (both EPA and DHA) and the recommended dose of vitamin D in combination with increased intake of olive oil taken by both the man and the woman for 6 weeks before IVF altered morphokinetic markers of early embryo development.

MATERIALS AND METHODS

The full protocol for the PREPARE trial has been previously published (18).

Recruitment

Full ethical approval (13/SC/0544) was granted from South Central (Oxford A) Research Ethics Committee (NRES) via the Integrated Research Application System (IRAS) and from the Research and Development Department (O+G0211) at University Hospital Southampton, United Kingdom. After approvals, couples awaiting IVF treatment who met the study inclusion criteria were invited to provide written consent to participate. These criteria were female age 18–41 years, body mass index (BMI) 18–32 kg/m^2, and the use of partner sperm. Exclusion criteria included more than two previous unsuccessful IVF cycles, low ovarian reserve indicated by an antimüllerian hormone (AMH) level <2 pmol/L (0.28 ng/mL; Beckman AMH Gen II), 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 (as defined by the U.K. Food Standards Agency) more than once a week. Those who were eligible and wished to participate were randomized to the study and control intervention groups.

The Intervention

The study group received olive oil for cooking, an olive oil–based spread, and a daily supplement drink enriched with EPA (800 mg), DHA (1,200 mg), and vitamin D (10 μg). The dose of EPA and DHA provided is consistent with doses used in other trials, can be obtained from foods (fatty fish), and is much greater than can be obtained from over-the-counter fish oil supplements. The dose of vitamin D used is in accordance with current U.K. recommendations for intake by pregnant women. 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. For the purpose of blinding, the drinks were provided by Smartfish (Oslo, Norway) in identical unmarked containers. The cooking oils and spreads were supermarket purchased and repackaged into identical unmarked containers.

The dietary intervention was to be taken for a minimum of 6 weeks preceding an IVF treatment cycle. The duration of the intervention was determined by two considerations: long enough to affect gamete maturation but not so long to be a burden or delay treatment, which would discourage participation in the study and compliance with the interventions. Support for this duration was provided by evidence that some lipid pools would have achieved maximal or near-maximal changes in EPA and DHA content within the 6-week period (19) and from rodent studies demonstrating that dietary manipulations within a very short window around the time of implantation can have profound effects on early development (20, 21).

Power Calculation and Sample Size

Recently, morphokinetic analysis of human embryos has shown the duration of key development phases to be correlated with standard conventional morphologic criteria, but to be more highly predictive of implantation potential (22, 23). In particular, the time taken to reach specific preimplantation developmental milestones has been shown to be predictive of embryo viability. A key morphokinetic marker of embryo viability has been shown to be the length of the second cell cycle (CC2; Fig. 1). A shortened CC2 has previously been associated with an increased likelihood that the embryo will develop to a blastocyst (24). In addition, embryos with a CC2 $<$11.9 hours (pooled SD 2.25 hours, but not normally distributed) have been reported to have an implantation rate of 35% compared with 28% when this key developmental step took $>$11.9 hours (22). Moreover, in a study correlating morphokinetic parameters with static morphology scoring, a CC2 $<$11.9 hours was shown to correlate with an overall increase in embryo score of 0.5 points on a 5-point scale (22). This is similar in magnitude to the impact on embryo morphology reported in a previous observational study, where a diet rich in omega-3 FAs was shown to be associated with altered embryo morphology markers when assessed on day 3 after fertilization (9). Given the evidence that CC2 may constitute a functional marker of embryo development, the primary end point of the present study was the difference in mean CC2 score of embryos generated by IVF after exposure of the couples to the study versus control diets. Based on work by Meseguer et al. (22), a 12% absolute difference (1.4 hours) in mean CC2, or effect size of 0.670, was considered to represent a developmentally significant effect. To show this with $\geq$80% power at $P<.05$, a nonparametric comparison (Wilcoxon test) indicated a requirement of 46 couples per group in the analysis. To allow for dropouts and failure to produce sufficient viable embryos, a further 20% were recruited. This required the randomization of at least 55 couples per group (110 in total).

Baseline Assessment

To address other possible factors determining embryo development, study participants were invited to complete a baseline preconception questionnaire establishing their characteristics and lifestyle, including age, ethnicity, education and occupation, exercise levels, alcohol and caffeine consumption, time spent outside, and sun cream use. Their BMI was measured and they completed the short Southampton Food Frequency Questionnaire (FFQ) (25) to assess the prudency of their diet. This comprised asking each individual about the frequency with which they consumed 20 food items (that best characterized a prudent dietary pattern from a 100-item interviewer-administered food frequency questionnaire used during the Southampton Women's Survey (25)). The more positive the score, the more prudent (or healthier) the described diet. Samples of blood were collected from the participants at the time of recruitment. Serum was used to measure vitamin D concentrations and red blood cells (RBCs) were used to measure FAs (see below).

Randomization

After collecting the baseline data, participating couples were randomized to one of the two intervention groups and were provided with a 6-week supply of the respective intervention components of drinks, oil, and spread in unmarked containers. Permuted block randomization was used with blocks of varying size and allocation concealment; stratification was performed at randomization for planned mode of fertilization: IVF or IVF-ICSI. The trial was double blinded: Neither the couples nor the research or clinical teams knew which arm of the study a couple had been assigned to. Unblinding was performed only after all of the couples had completed the dietary intervention and annotation of all of the embryos had been performed.

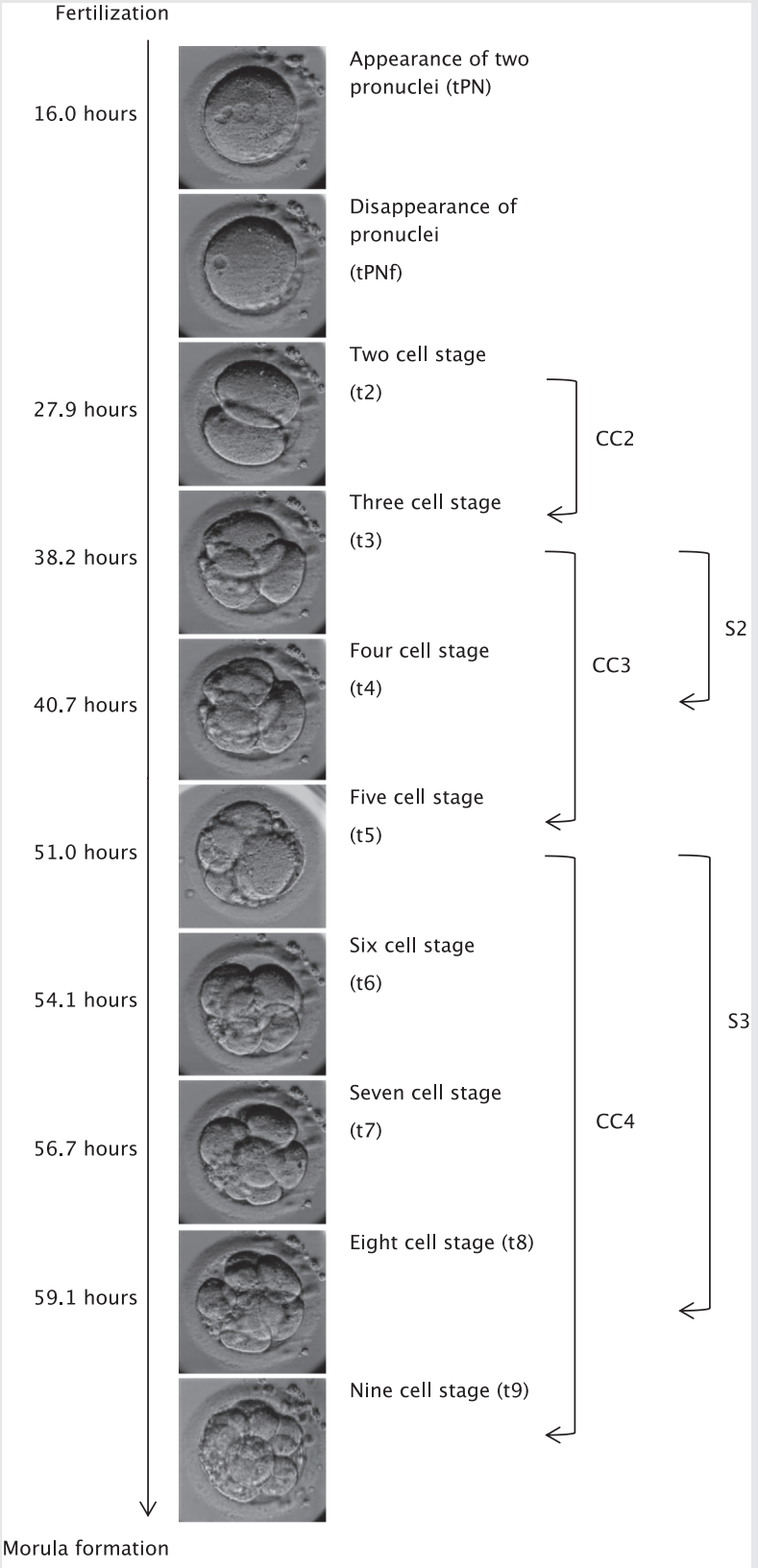
Compliance

Compliance was monitored by weekly communication (telephone calls or e-mail) by the research team.

IVF Cycle

Women embarking on the study underwent ovarian stimulation according to the standard protocols used by the IVF unit with the use of gonadotropins and cotreatment with a GnRH agonist or antagonist to prevent premature luteinization. Oocyte retrieval was performed 36 hours after triggering of final oocyte maturation by a single subcutaneous dose of

FIGURE 1



Development of the embryo from the point of fertilization of the oocyte to morula formation, with average times for the general population (46). Images taken from PREPARE patient 157.

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hCG or GnRH agonist. IVF alone or ICSI was performed where clinically indicated. On the day of oocyte retrieval (after ~6 weeks of dietary study intervention or control), the initial lifestyle questionnaire was readministered and a further blood sample was collected from the participants.

Embryo Culture

The embryos were cultured in sequential G-series culture media (Vitrolife) in a validated time-lapse incubator (EmbryoScope D; Vitrolife) in 6% CO_2 and 5% O_2 at 37°C, according to the standard laboratory protocol. During incubation, seven focal-plane images were taken every 10 minutes, generating 42 plane focal images every hour, and these were analyzed according to morphologic and morphokinetic markers (26). The morphokinetic markers of 750 embryos (356 in the study group and 394 in the control group) were annotated by embryologists blind to the study group, and the end point parameters were calculated. The morphokinetic markers were measured from the time of insemination or the start of the injection for ICSI. Standard time points were annotated and calculated (including the time for the second [CC2], third [CC3], and fourth [CC4] cell cycles and the synchrony of the second [S2] and third [S3] cell cycles). These morphokinetic markers were then used to calculate the day 3 and day 5 Known Implantation Data Scores (KIDScores) in accordance with published (27) and unpublished (28) algorithms, respectively. The embryos were then transferred, cryopreserved, or destroyed according to the clinic's standard operating procedures. The embryo or embryos with the highest morphologic score based on validated criteria (Gardner) (29) were transferred; embryos were cryopreserved if they were grade 3 or higher with a Gardner a- or b-grade trophectoderm and inner cell mass (30).

Primary and Secondary End Points

The primary end point for the study was one of a series of validated morphokinetic parameters of healthy embryo development, namely the time taken for completion of CC2. Secondary end points included blood measurements of EPA, DHA, and vitamin D and additional validated parameters of embryo development, such as time to complete CC3 and CC4, and S2 and S3, associated with increased chance of development to a blastocyst (24, 31, 32), implantation (22, 24, 33, 34), and clinical pregnancy (24). In addition, the day 3 and day 5 KIDScores were calculated and pregnancy data were analyzed. Other studies have examined fertilization rates as the primary outcome; however, using morphokinetic markers and algorithms enabled the PREPARE trial to study the effect of the intervention on the development of the embryo.

Analysis of Fatty Acids in Red Blood Cells

The fatty acid content of RBCs was measured by means of gas chromatography, allowing the separation and identification of 19 fatty acids, including EPA and DHA. The protocol used was as described elsewhere (35).

Analysis of Serum Vitamin D Concentration

Serum vitamin D concentrations were determined by means of liquid chromatography/tandem mass spectrometry (Waters). The pathology laboratory that undertook the analysis, University Hospital Southampton NHS Foundation Trust, is a member of the Vitamin D External Quality Assurance Scheme.

Statistical Analysis

Results are reported as mean ± SD unless otherwise stated. Differences between the sociodemographic characteristics of participants in the two groups were analyzed with the use of analysis of variance (ANOVA). Characteristics that were not normally distributed and were scalar were adjusted by log transforming and then included in the ANOVA, which enabled a more powerful statistical analysis than with the use of nonparametric tests.

ANOVA was also used to compare the levels of FAs and vitamin D in the blood of the participants in the two groups. Results that were not normally distributed were log transformed and then included in the analysis.

A mixed-effects model was used to analyze the effect of the intervention on the morphokinetic markers of embryo development. A random effect was fitted for each couple and a fixed effect for treatment and the methodology (i.e., IVF or IVF-ICSI) used to inseminate the embryo. Treatment effects are summarized by the regression coefficient and its standard error. The intraclass correlation coefficient measures the within-to-between couple variation.

RESULTS

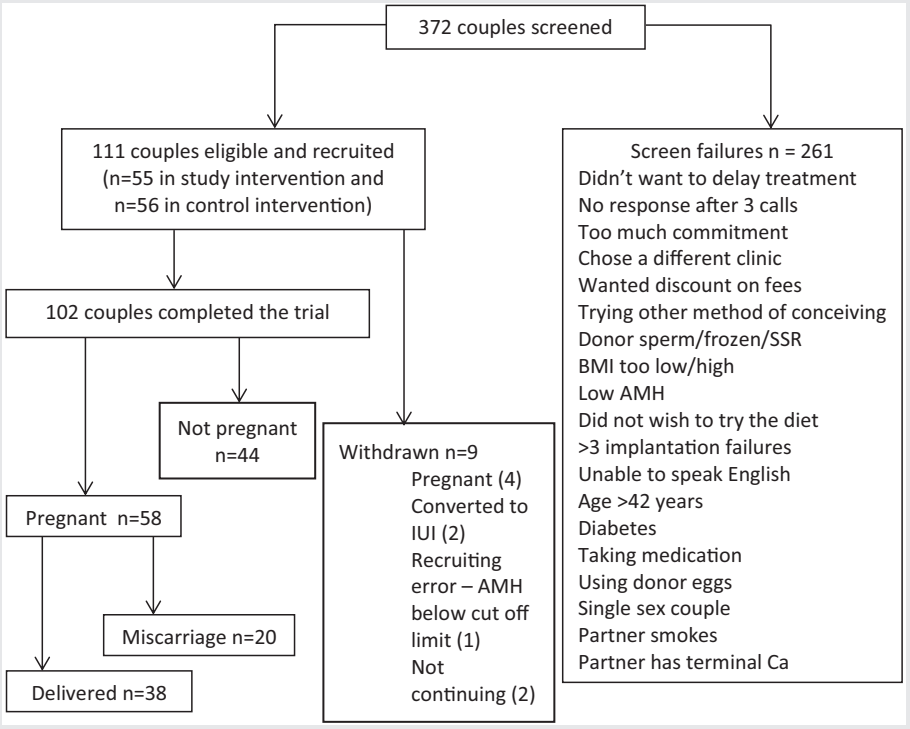
Demographic Data

One hundred eleven couples were recruited from February 2014 to November 2015 (Fig. 2). Of these, 102 completed the trial and had at least one embryo analyzed, four became pregnant before their treatment, two had cycles that were converted to intrauterine insemination after ovarian stimulation, one was a recruiting error, and two had health issues leading to withdrawal.

More than 90% of study participants were of white European ethnicity, representative of the local ethnic profile. The mean ages of the participants in the trial were 33.4 ± 4.2 years for the women and 36.0 ± 5.5 years for the men. There was no significant difference in BMI, prudent diet score, quantity of alcohol and caffeine consumed, and number of hours of exercise per week between the study group and the control group (Table 1). Monitoring of these markers showed no evidence that participants instituted other lifestyle changes during the study period.

At the trial commencement, 25 women (23%) and 61 men (55%) reported not taking any supplements. 52 women (47%) and 32 men (29%) were taking a multivitamin supplement (specific to either conception or pregnancy), and 6% of women and 11% of men were taking an omega-3 supplement (either in conjunction with a multivitamin or on its own). All omega-3 supplements were of a sufficiently low dose (<200 mg EPA and DHA) to render these patients eligible for inclusion in the trial.

FIGURE 2



CONSORT diagram for the trial. AMH = antimüllerian hormone; BMI = body mass index; IUI = intrauterine insemination; SSR.

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Compliance

Sixty-two percent of the women and 50% of the men reported full compliance with their allocated intervention. There was no statistical difference in compliance between the study group and the control group in either the women ($P=.799$) or the men ($P=.089$). The median compliances to the drinks were 100.0% (interquartile range [IQR] 97.2%–100.0%) for women and 99.3% (95.3%–100.0%) for men.

Red Blood Cell Fatty Acids and Serum Vitamin D

There were no significant differences in the preintervention levels of any of the 19 different FAs measured in RBCs between the study and control groups in either the women or the men (data for EPA and DHA presented in Table 1).

Among both women and men, both EPA and DHA increased in the study group. There were increases in EPA of 2.30% (2.80%–3.21%) in women and 2.51% (2.30%–2.74%) in men and in DHA of 2.80% (2.57%–3.03%) in

TABLE 1

Dietary and lifestyle characteristics of the women and men recruited into the study.

	Women			Men		
Characteristic	**Intervention (n = 55)**	**Control (n = 56)**	***P* value**	**Intervention (n = 55)**	**Control (n = 56)**	***P* value**
Age (y)	33.3 ± 4.1	33.4 ± 4.3	.9	35.6 ± 5.8	36.4 ± 5.3	.4
Body mass index (kg/m^2)	24.3 ± 3.1	25.0 ± 3.9	.3	26.8 ± 4.1	27.2 ± 4.0	.6
Prudent diet score	0.02 ± 0.90	0.05 ± 1.13	.9	0.01 ± 0.92	−0.11 ± 1.08	.5
Alcohol consumption (units/wk)	4.0 (3.0–6.0)	4.0 (2.3–6.8)	.8	8.0 (4.3–11.8)	6.0 (3.8–12.0)	.1
Caffeine consumption (mg/d)	84.0 (31.5–157.5)	108.0 (53.0–186.0)	.5	162.0 (75.5–337.5)	189.5 (107.3–328.5)	.8
Exercise (h/wk)	3.0 (2.0–4.0)	2.0 (1.0–4.8)	.5	4.0 (2.1–6.0)	5.0 (1.9–8.1)	.9
Eicosapentaenoic acid[a] (% of RBC)	0.97 ± 1.36	0.96 ± 1.35	.7	0.94 ± 1.36	0.91 ± 1.36	.6
Docosahexaenoic acid (% of RBC)	5.16 ± 0.98	5.03 ± 1.10	.5	4.66 ± 1.09	4.51 ± 1.14	.5
Total vitamin D (nmol/L)	74.33 ± 29.41	71.62 ± 24.69	.6	67.48 ± 28.01	68.11 ± 27.11	.9

Note: Values are presented as mean ± SD or median (interquartile range). RBC = red blood cells.
[a] Distribution skewed, so data were log transformed before analysis.

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women and 2.42% (2.12%–2.71%) in men in the study group (all $P<.001$). No significant increases were observed in the control group except a 0.82% (0.87%–0.97%) increase in EPA in women ($P=.002$).

At study entry, 7% of men and 4% of women had a total serum vitamin D concentration <25 nmol/L, indicative of deficiency. A further 20% of men and 19% of women had borderline deficiency (25–50 nmol/L). As expected, the season of recruitment affected the vitamin D concentration measured; men and women who were recruited in the summer (77.75 ± 25.01 nmol/L and 82.1 ± 26.063 nmol/L, respectively) had higher mean total concentrations than those recruited in the winter (49.07 ± 22.51 nmol/L and 49.47 ± 18.42 nmol/L; $P<.001$ and $P=.001$, respectively).

At the end of the treatment period, men and women in the study group had higher serum total vitamin D concentrations than those in the control group (geometric means 154.63 ± 1.56 nmol/L vs. 68.50 ± 1.51 nmol/L in women [$P<.001$] and 137.31 ± 1.54 nmol/L vs. 66.57 ± 1.49 nmol/L in men [$P<.001$]).

Analysis of Embryo Development

There was no difference between the study group and the control group in the number of oocytes retrieved (medians 10.00 [IQR 6.00–15.75], and 11.00 [7.50–18.50]; $P=.500$) or in the number of normally fertilized embryos obtained (medians 6.00 [2.00–9.00] and 6.00 [3.50–12.00]; $P=.299$).

Seven hundred fifty embryos were analyzed (356 in the study group and 394 in the control group). Of these, 742 embryos cleaved to the 2-cell stage (351/356 [98.6%] vs. 391/394 [99.2%]; $P=.392$), 719 cleaved to the 4-cell stage (344/356 [96.6%] vs. 375/394 [95.2%]; $P=.319$) and 610 to the 8-cell stage (295/356 [82.9%] vs. 315/394 [79.9%]; $P=.306$). Furthermore, 487 embryos formed a blastocyst (231/356, 64.9% vs. 256/394, 65.0%, $P=.980$).

Table 2 presents the median and quartile values of the morphokinetic markers for the study and control groups. The markers are expressed in standardized form and the treatment effects are presented in Table 2. CC2 times were 0.04 standard deviations (95% CI −0.16 to 0.24) shorter in the study group than in the control group, but the two groups were not significantly different ($P=.71$). There were statistically significant reductions in CC4 and S3 times ($P<.001$ and $P=.02$, respectively) and an increase in KIDScore on day 3 ($P=.05$) in the study group compared with the control group (Table 2). There was no significant difference observed in the amount of fragmentation, blastomere evenness, or multinucleation on day 3 between the two groups. No difference between groups was observed in the time it took the embryos to form a morula, to start blastulation, to form a blastocyst, to form an expanded blastocyst, or to form a hatching blastocyst (Table 2). There was also no difference between the average number of blastocysts formed (medians 4.00 [IQR 0.00–6.00] vs. 3.00 [0.00–9.00]; $P=.619$) or the average number of blastocysts suitable for cryopreservation (medians 3.00 [0.00–4.00] vs. 2.00 [0.00–5.00]; $P=.823$) between the study group and the control group, respectively.

Pregnancy Rates

The study was not adequately powered to look at pregnancy rates, and no difference was observed between the two groups, either in the first cycle after the trial (30/53 in the study group, 28/49 in the control group; $P=.956$) or per embryo transferred (fresh and frozen) to date (169 embryos, with pregnancy rates of 41/78 in the study group and 55/91 in the control group; $P=.303$). Furthermore, there was no difference in live birth rates: 42% (22/53) in the study group and 33% (16/49) in the control group following the first cycle ($P=.355$). Live births per embryo transferred to date were 27/78 in the study group and 28/91 in the control group ($P=.595$).

TABLE 2

Morphokinetic markers of embryo development according to intervention group.

	Study intervention group			Control intervention group			Mixed model analysis			
Morphokinetic marker	n	Median	IQR	n	Median	IQR	Treatment effect	95% CI	*P* value	ICC
CC2	349	11.51 h	10.51–12.34	381	11.51 h	10.67–12.51	−0.04	−0.24 to 0.16	.71	0.10
CC3	338	13.50 h	11.96–15.02	368	13.01 h	11.51–15.34	0.05	−0.19 to 0.28	.70	0.18
CC4	283	19.34 h	16.79–23.07	298	21.94 h	18.51–26.48	−0.45	−0.69 to −0.21	<.001	0.15
S2	344	0.50 h	0.17–1.33	375	0.67 h	0.33–1.80	−0.14	−0.31 to 0.03	.11	0.05
S3	295	4.67 h	2.17–14.34	315	5.84 h	2.50–18.67	−0.23	−0.42 to −0.03	.02	0.06
tM	298	94.89 h	88.04–102.00	313	94.52 h	88.23–100.98	0.03	−0.30 to 0.35	.87	0.38
tSB	282	100.91 h	93.61–108.87	298	100.04 h	94.55–107.15	−0.01	−0.32 to 0.30	.95	0.34
tB	231	109.29 h	102.25–118.98	256	110.72 h	104.06–118.37	−0.11	−0.41 to 0.18	.45	0.26
tEB	185	116.83 h	109.95–129.87	192	118.12 h	112.06–127.27	−0.05	−0.39 to 0.29	.77	0.31
tHB	98	119.41 h	112.19–132.85	78	123.49 h	114.81–131.89	0.01	−0.45 to 0.47	.96	0.47
KIDScore D3	340	4	3–5	376	4	2–5	0.18	0.00 to 0.37	.05	0.17
KIDScore D5	323	2	1–5	355	2	1–4	0.35	−0.08 to 0.77	.11	0.21

Note: Treatment effect set as "Intervention − Control" with markers in standardized (z-score) form. CC2 = time to complete second cell cycle; CC3 = time to complete third cell cycle; CC4 = time to complete fourth cell cycle; CI = confidence interval; ICC = intraclass correlation coefficient; IQR = interquartile range; S2 = synchrony of the second cell cycle; S3 = synchrony of the third cell cycle; tB = time to form a blastocyst; tEB = time to form an expanded blastocyst; tHB = time to form a hatching blastocyst; tM = time to form a morula; tSB = time to start blastulation.

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DISCUSSION

To our knowledge, this is the first randomized controlled trial using IVF as a model to study the impact of a preconceptional dietary intervention on markers of preimplantation human embryo development. The dietary intervention did not reveal a significant effect on CC2, but the effect on other key morphokinetic markers indicated a potentially positive influence of the study intervention on the developing embryo. Specifically, CC4 and S3 were shortened in embryos derived from couples who had taken a 6-week intervention of high doses of omega-3 FAs and the recommended vitamin D intake along with olive oil. The observed impact of the dietary intervention on CC4 and S3, as opposed to the earlier morphokinetic markers (including CC2), may reflect a cumulative amplification of the effect over time. A difference in the day 3 KIDScore was also observed in the study group, demonstrating an overall improvement in the morphokinetic markers of embryo quality. Interestingly, a difference in time to blastocyst formation was not observed, which might suggest that blastocysts with a slower CC4 have fewer cells in the trophectoderm and inner cell mass; this needs further research but was beyond the scope of this study. Furthermore, this study did not enable us to determine whether it was an effect of the dietary intervention on the female or male gametes or an additive effect that resulted in the change in embryo development.

Although the focus of this study was the impact of a dietary supplement on markers of embryo development rather than clinical outcomes of IVF, a considerable body of evidence from observational studies indicates that the reported findings may have implications for clinical practice. A shortened CC4 has been shown to be positively predictive of continuing development to the blastocyst stage and of achieving a clinical pregnancy (24). Similarly, a relationship has been demonstrated between a shortened S3 and the chance that the embryo will develop to the blastocyst stage (24). It has therefore been postulated that a diet rich in omega-3 FAs, vitamin D, and olive oil may increase pregnancy rates in couples undergoing assisted reproductive technologies (ART). This notion was supported by an observational study reporting that higher serum omega-3 FAs in women undergoing IVF were associated with an increased chance of clinical pregnancy and live birth (10). Furthermore, a recent publication examining protein intake and fertility treatment outcome demonstrated a positive correlation between intake of fish (the main dietary source of bioactive omega-3 FAs) and live birth rate (36).

Vitamin D levels have been implicated as a factor affecting endometrial receptivity (37). To date, benefit of vitamin D in ART patients has been demonstrated in couples who were depleted before starting the supplements (3). In the PREPARE trial, fewer than 5% of the women were depleted at recruitment, compared with previously cited proportions of 35%–45% (38). The lack of deficiency may mean it is possible that the full effect of the vitamin D supplementation was not elicited; owing to the small numbers of deficient patients it was not possible to do a subanalysis. A previous observational study reported an association between serum vitamin D levels during ovarian stimulation in women who were not deficient and a higher fertilization rate, but no correlation with clinical pregnancy or live birth rate was observed (39). Olive oil was included in the trial because it represents a key element of the Mediterranean diet. A recent study showed that when taken with a high-fish diet in the periconceptional period, olive oil accelerated embryo development in the 6th to 11th weeks of gestation (40).

It should also be recognized that the trial design meant that the individual components of the study intervention resulting in the alteration of the morphokinetic markers could not be determined; it is possible that the benefit seen might be due to either omega-3 FAs, vitamin D, or olive oil individually. Additional randomized interventional studies are required to confirm or refute the proposed benefits of omega-3 FAs, vitamin D, and olive oil, independently or synergistically, for improving fertility outcomes, but the present study indicates that even a relatively short and thus well tolerated intervention may have beneficial effects.

This study also demonstrated the feasibility of recruiting couples who are trying to conceive into randomized controlled nutritional intervention trials. Compliance was high and biochemical markers of omega-3 FA status and vitamin D in blood indicated significant enrichment in the both men and women in the study group, consistent with good compliance.

Some limitations of this trial should be noted. The duration of the intervention was 6 weeks, which might have limited the effect on embryo development because it was shorter than the reported duration of oocyte and sperm maturation, which is thought to take ~3 months (41) and 72 days (42), respectively. However, although human data are lacking, the latter phases of gamete maturation have been shown to be sensitive to environmental factors (43), and as outlined previously the duration of the intervention was supported by studies in mice that have demonstrated a remarkable impact of a very short-term preconceptional dietary intervention (3.5 days) on in utero growth trajectories and even behavior development (44). Moreover, RBC levels of omega-3 FAs and serum vitamin D were increased by 6 weeks in the present study, showing that the trial intervention was effective in altering the in vivo nutritional milieu.

The FA profile of the women before the intervention was similar to that reported in a previous fish oil supplementation study in pregnancy (45). This suggests that the RBC fatty acid profile of the studied women is representative of the wider female population of this age. However, it should be noted that the study population was predominantly white European and care should be taken when extrapolating the evidence to those from other ethnic backgrounds.

CONCLUSION

In conclusion, this study provides further confirmation that preconceptional nutritional status can affect embryo development. Although the dietary intervention did not result in an alteration in the CC2 rate, omega-3 FAs and vitamin D in the blood were increased and a demonstrable impact on the development of the preimplantation embryo in the CC4

and S3 rates was observed. Further intervention studies of sufficient power are required to determine the optimal duration of a preconceptional dietary intervention containing omega-3 FAs, vitamin D, and olive oil that might affect clinical outcomes.

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