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Mediterranean diet and outcomes of assisted reproduction: an Italian cohort study.

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Short title: Mediterranean diet and ART outcomes

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Condensation

In an Italian population of women undergoing ART, no clear association was observed between adherence to a Mediterranean diet and successful IVF.

AJOG at a Glance

A. Why was the study conducted? To assess the effect of diet on reproductive success, in a group of women undergoing assisted reproduction

B. What are the key findings? No consistent associations were observed between adherence to a Mediterranean diet and successful obstetrics outcomes.

C. What does this study add to what is already known? This study observed a protective effect of intermediate adherence score to Mediterranean diet on oocyte number and clinical pregnancy in women older than 35 years, but no effect on live birth.

Keywords: assisted reproduction techniques; Mediterranean diet; cohort study; life style.

Abstract

Background. Detrimental lifestyle habits have been indicated as potential causes of reduced fertility. Recently, studies have suggested an association between healthy diets and increased live birth rates after assisted reproduction techniques (ART). However, the issue remains under debate, and evidence is still accumulating.

Objective. To study the relation between Mediterranean Diet and outcomes of ART in subfertile couples in an Italian population.

Study design. Prospective cohort study, conducted in an Italian Fertility Clinic. Couples undergoing *in vitro* fertilization (IVF) were interviewed on the day of oocyte retrieval to obtain information on personal and health history, lifestyle habits and diet. Adherence to Mediterranean Diet was evaluated using a Mediterranean Diet Score (MDS). Relative risks (RR) and 95% confidence intervals (CI) for embryo transfer, clinical pregnancy, and live birth were calculated. - Potential confounders were included in the equation model.

Results: Among 474 women (mean age 36.6 years, range 27-45), 414 (87.3%) performed embryo transfer, 150 (31.6%) had clinical pregnancies, 117 (24.7%) had live births.

In a model including the potential confounders (age, leisure physical activity, body mass index, smoking, daily calories intake and previous failed IVF cycles), findings showed that MDS was not significantly associated with IVF outcomes. Adjusted analyses were performed in strata of age, previous ART cycles, and reasons for infertility, with consistent findings. The only exception was observed in women >35 years old with intermediate MDS, who showed a lower risk of not achieving clinical pregnancy (Adjusted RR 0.84, 95% CI 0.71-1.00, p=0.049).

Conclusions. No clear association was observed between adherence to a Mediterranean diet and successful IVF.

INTRODUCTION

Infertility is a medical condition recognized by World Health Organization[1] and defined as "the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourses". It affects up to 25 million people in Europe. Male and female causes of infertility are identified in 20-30% and 20-35% couples, respectively. Both partners are involved in 25-40% of cases and in 10-20% of cases no cause is found. [2]

Besides non modifiable factors (parental age, low ovarian reserve), detrimental lifestyle habits have been suggested as potential causes of reduced fertility [3–5]. In particular, over the last decade literature on the relation between diet and fertility has expanded. A recent review [6] summarized the findings of several studies, identifying few clear patterns. Focusing on the outcome of assisted reproduction techniques (ART), an adequate supply in folic acid [7] and isoflavones [8] were shown to be associated with increased live birth rates, as well as healthy diets [9]. Conversely, the role of diet patterns and food groups remains under debate, and evidence is still accumulating.

In this paper, to provide further information on this issue, we have analyzed the relation between Mediterranean diet and its components, and *in vitro* fertilization (IVF) outcome, using data from a cohort study conducted in an Italian Fertility center.

MATERIALS AND METHODS

From September 2014 to December 2016, in randomly selected days, subfertile couples, presenting for evaluation to the Fertility Unit of Fondazione IRCCS Ca' Granda Ospedale Maggiore, Policlinico located in Milan (Northern Italy), and eligible for IVF, were invited to participate into an ongoing prospective cohort study on the role of lifestyle habits and diet on ART outcome. The study protocol was approved by the Ethical Review Board of Fondazione IRCCS Ca' Granda Ospedale Maggiore, Policlinico (Milan, Italy). All procedures were in accord with the Helsinki Declaration and all participants provided written informed consent.

Study participation was proposed during the diagnostic phase and couples were interviewed on the day of oocytes retrieval. The time interval between the proposal of the study and the interview was generally less than one month. Using a standard questionnaire to obtain information on general socio-demographic characteristics, health history and habits (including smoking, physical activity, alcohol intake and methylxanthine-containing beverages consumption), centrally trained personnel interviewed both partners of couples who agreed to participate. Couples that did not speak fluently Italian were excluded. The present study reported exclusively on evidence obtained from the female partner in relation to the outcome of the first cycle after the interview.

The overall participation rate was close to 95%. This high participation rate was mainly due to the fact that couples were interviewed during the down time spent in the hospital for the procedure (that commonly lasts from 3 to 6 hours), and to the non-sensitive character of the questions.

Information on diet was based on a reproducible and valid food frequency questionnaire (FFQ), . Patients were asked to report about their usual weekly food consumption in the last year. The FFQ includes the average weekly consumption of 78 food items or food groups (such as the major sources of animal fats – i.e. red meat, fish, milk, cheese, ham, salami; folates, vitamins – vegetables and fruit; carbohydrates - pasta and bread consumption, cake and sweets such as chocolate) and beverages. [10–12] Intakes lower than once per week, but at least once per month, was coded 0.5 per week. Seasonal consumption was also considered (weekly consumption of vegetables/fruits available in limited periods during the year, weighted for months of consumption). Energy and mineral, macro- and micronutrient intake was estimated using the most recent update of an Italian food consumption database [13].

The FFQ had been previously validated using two 7-day dietary records. For most nutrients correlation coefficients were equal or greater than 0.60, indicating a fair (e.g. animal protein 0.64, vegetable protein 0.60, available carbohydrates 0.64, starch 0.72, fiber 0.53, total fat 0.50) [12]. The FFQ also showed good reproducibility with most correlation coefficient for specific food items[10] and nutrients, [11] ranging between 0.60 and 0.80.

Folate supplementation was not recorded because in our centre it was prescribed to all patients planning a pregnancy.

Information on alcohol intake was collected as usual weekly consumption (1 unit=125 mL wine or 330 mL beer or 30 mL spirits, all containing approximately 12.5 g of ethanol). Intakes lower than one unit/week were codified as 0.5. Total alcohol intake, expressed in grams of ethanol per day (g/day), was computed as the sum of all reported alcoholic beverages. “Never drinkers” were patients who abstained from drinking lifelong; “Ex-drinkers” were individuals who had abstained from drinking for at least 12 months at the time of interview. For the purpose of this study, we considered these women in the same category “Abstainers”. The reproducibility and validity of alcohol consumption had been tested and showed satisfactory results.[14]

Further, questions included information on the average number of cups per day of coffee and other methylxanthine-containing beverages (tea, cocoa and decaffeinated coffee). Caffeine intake from coffee (60 mg per cup), cappuccino (75 mg per cup), tea (45 mg per cup), decaffeinated coffee (4 mg per cup) and chocolate (6 mg/10 g) was calculated [15].

A woman was considered a smoker if she had smoked more than one cigarette/day for at least one year; an ex-smoker if she had smoked more than one cigarette/day for at least one year, but had stopped more than one year before the interview, and a non-smoker if she had never smoked more than one cigarette/day.

Occupational physical activity (PA) was described as heavy (or very heavy), light/moderate, mainly standing or mainly sitting. Leisure PA was recorded in term of hours/week: <2, 2 to 4, ≥5. No information was collected about intensity or type of leisure PA.

Mediterranean Diet Score (MDS). The adherence to the Mediterranean diet was assessed through an a priori score (Mediterranean diet score, MDS), developed by Trichopoulou and colleagues [16] and modified to also include potatoes. It included nine dietary components: fruit, vegetables, cereals (including bread and potatoes), legumes, fish, monounsaturated/saturated fatty acid ratio, dairy products, meat (including meat products) and alcoholic beverages. For each study subject and for each score component, a value of 0 or 1 was attributed as follows: for components frequently consumed in the traditional Mediterranean diet (i.e., fruit, vegetables, cereals, legumes, fish and high monounsaturated/saturated fatty acid ratio), subjects were assigned a value of 1 if they had a consumption above or equal to the study-specific median among controls and 0 otherwise; for components less frequently consumed in the Mediterranean diet (i.e., dairy and meat products), women with a consumption below the study-specific median were assigned a value of 1 and 0 otherwise (Table 1S). For alcohol, 1 point was attributed to women consuming 5 g to less than 25 g of ethanol per day and 0 otherwise. We then calculated the MDS adding up the points for each of the nine individual binary components; thus, the score varied between 0 and 9, the higher the score the stronger the adherence to Mediterranean diet.

Patients were managed according to a standardized clinical protocol as reported in details elsewhere [17,18]. Briefly, regimens of hyper-stimulation and drugs dosages were decided based on clinical characteristics and biomarkers of ovarian reserve. In case of hypo-response or abnormal follicular growth, the cycle could be canceled before ovum pick up. A freeze-all strategy was conversely preferred in case of hyper-response. Oocytes retrieval was performed 36 h after ovulation triggering and embryo transfer was generally performed two to five days after oocyte insemination according to embryo quantity and quality. The choice between conventional IVF or Intra-Cytoplasmatic Sperm Injection (ICSI) was made based on semen characteristics. Good quality oocytes were those in metaphase I-II for IVF and metaphase II for ICSI. Using embryological criteria, morphological data based on stage-appropriate number of evenly sized blastomere, absence/presence of multinucleation and pattern of fragmentation were recorded after 48 to 72 hours of activation (day 2 or 3). Embryos belonging to grade 1 or 2 (fragmentation less than 10% with equal-sized blastomeres) were classified as “good quality” ones. [19]

However, embryo transfer was postponed through embryo vitrification in the following conditions: 1) if the number of retrieved oocytes exceeded 15 or if serum estradiol level exceeded 4000 pg/ml in order to reduce the incidence of Ovarian Hyper-stimulation Syndrome (OHSS); 2) if serum progesterone exceeded 1500 pg/ml at the time of ovulation triggering. Viable non-replaced embryos were vitrified mostly at the blastocyst stage. Women with frozen embryos were scheduled for natural cycle embryo transfer if they referred regular menstrual cycles and a mean cycle length between 24 and 35 days. Embryo transfer in the natural cycle was performed 4–6 days after LH surge (detected with the use of urinary sticks) according to the embryo age and no luteal phase support was given. Hormone replacement treatment was prescribed if women had irregular menstrual cycles or if the monitoring of the natural cycle failed [18].

Serum hCG assessment to detect pregnancy was performed+14/16 days after ovulation triggering or LH surge. Women with positive hCG values underwent a transvaginal sonography three weeks later. Clinical pregnancy was defined as the presence of at least one intrauterine gestational sac. In this analysis, we considered as outcome the best one obtained using the oocytes retrieved in the cycle immediately following the interview, For example, if a woman did not achieve a pregnancy with a fresh embryo transfer, but subsequently a frozen embryo from the same cycle led to a clinical pregnancy, we considered the clinical pregnancy as the main outcome.

All clinical information (including infertility diagnoses) was collected from medical records.

Statistical analyses

Clinical pregnancy was considered the main objective of the study. Considering a 30% of pregnancy rate per cycle, as usual in our Fertility Centre, this study was powered to detect a 1.5 increase of risk in the highest tertile of intake as compared to the lowest (α=0.05, β=0.80).

Multiple outcomes were considered in this analysis: 1. Number of retrieved good quality oocytes; 2. Number of good quality embryos; 3. Embryo transfer; 4. Clinical pregnancy; 5: Live birth.

Categorical variables were described as frequency (N) and percentage (%) and compared using the Pearson or Mantel-Haenzsel (MH) chi-square, as appropriate. Continuous variables were described as mean and standard deviation (SD) if normally distributed, or median and interquartile range

(IQR) if not normally distributed, and analyzed using analysis of variance and Kruskal-Wallis test respectively.

In order to perform a multivariate analysis including potential confounders, non-normal (skewed) distributed numbers of good quality oocytes were square-root transformed and included in a general linear model. Adjusted medians and 95% confidence interval (CI) were calculated back-transforming the adjusted means and their 95% CIs. In the model, we included as potential confounders variables associated to MDS or number of good quality oocytes or embryos at univariate analysis.

We estimated relative risks (RRs) of each outcome and corresponding 95% CIs in categories of MDS (approximate tertiles) in the year before the interview. To account for potential confounders, we included terms for variables that were associated with MDS and/or with at least one IVF outcome, in the multiple log-binomial regression model (as indicated in table footnotes).

All the analyses were performed using the SAS software, version 9.4 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

During the study period, out of 501 eligible women, 27 (5.4%) did not provide complete information about their diet and were excluded. Comparing women included and excluded from the present analysis, we did not observe any significant difference in term of socio-demographic characteristics and ART outcomes. In the remaining 474 included women, mean age was 36.6 years (standard deviation, SD, 3.6, range 27-45) and mean body mass index (BMI) was 22.3 kg/m^2 (SD 4.0, range 17.0-42.0). Twenty-nine women were obese (BMI > 30 kg/m^2).

Table 1 shows the characteristics of considered women according to adherence to MDS. No significant association was observed between MDS and age, BMI, education, occupational physical activity, cause of infertility, and previous ART cycles. MDS was related to daily calories intake and leisure physical activity.

In 474 women, 414/474 (87.3%) cycles resulted in embryo transfer, 150/474 (31.6%) in clinical pregnancies, 117/474 (24.7%) in live births. Out of 33 clinical pregnancies not resulting in live birth, 32 ended in miscarriage and one in induced abortion.

Age was the main risk factor for IVF failure: as compared to women aged less than 35, RR for not achieving embryo transfer was 2.09 (95% CI 1.03-4.22) for women aged 35-39 and 2.40 (95% CI 1.12-5.13) for those aged ≥40 years. The corresponding figures were 1.19 (95% CI 1.00-1.40) and 1.41 (95% CI 1.18-1.67) for clinical pregnancy; 1.20 (95% CI 1.03-1.39) and 1.37 (95% CI 1.18-1.59) for live birth. After adjusting for age, previous IVF cycles were associated to higher risk of unavailability of embryos for transfer (RR 1.79, 95% CI 1.05-3.03) and exercising for ≥5 hour per week to higher risk of non achieving clinical pregnancy (RR 1.25, 95% CI 1.05-1.49) as compared to <2 hours per week.

Table 2 shows the relation between MDS level and clinical results. Numbers of good quality oocytes or embryos were not significantly different among classes of MDS. Since we observed a borderline trend for increasing number of good quality embryos, we performed a supplemental analysis to evaluate the ratio between good quality embryos and good quality oocytes. We found that, despite the adjusted median increased through MDS category (0.25, 0.29 and 0.32 in 0-3, 4-5 and 6-9 MDS), no significant difference exists (chi-square for trend 1.45, p=0.15).

Adjusted RRs for IVF failure at each step (embryo transfer, clinical pregnancy, live birth) were calculated. All analyses included age, leisure PA, daily calories intake, previous ART cycles, smoking habits, obesity and daily calories intake in the model.

Analyses were also performed in strata of age (Table 2), previous ART cycles, and cause for infertility (data not shown): findings consistently showed that MDS was not significantly associated with IVF outcomes, although a slightly lower risk of adverse outcome was consistently observed in the MDS 4-5 group. A lower risk of not achieving clinical pregnancy in women aged more than 35 years, with an intermediate level of MDS, was of borderline significance (Adjusted RR 0.84, 95% CI 0.71-1.01, p=0.07).

We rerun the analyses excluding alcohol intake from the MDS, and including it in the regression model.

Adjusted median oocytes were 5.4 (95% CI 3.0-8.7), 6.4 (95% CI 3.7-9.7) and 5.4 (95% CI 2.9-8.6) in the 0-3, 4-5 and 6-8 category, respectively. Whereas the difference between 0-3 and 4-5 MDS with alcohol was of borderline significance in the main analysis, after excluding alcohol from the score we found that the difference became statistically significant (p=0.043). However, alcohol intake *per se*, included as confounder in the model, was not significantly associated with number of good quality retrieved oocytes. Good quality embryos showed a significant increase in category of MDS excluding alcohol from the score: adjusted medians were 1.3 (95% CI 0.9-1.9), 1.8 (95% CI 1.3-2.4) and 1.9 (95% CI 1.3-2.6), in 0-3, 4-5 and 6-8 category respectively, with a significant trend (chi-square 2.16, p=0.03). However, this did not translate into better pregnancy outcome. One hundred fifty five women (32.7%) had 0-3, 203 (42.8%) 4-5 and 116 (24.5%) 6-8 MDS. The results were similar: using the lower MDS category as the reference, RR for failure in embryo transfer was 0.79 (95% CI 0.45-1.39) in 4-5 and 0.91 (95% CI 0.51-1.63) in 6-8 MDS. The correspondent figures for failure in achieving clinical pregnancy were 0.96 (95% CI 0.89-1.05) and 0.96 (95% CI 0.88-1.06) and for not achieving live birth 0.99 (95% CI 0.90-1.10) and 0.97 (95% CI 0.89-1.09). Alcohol intake was not associated with any of these outcomes.

Finally, we analyzed the association between food groups included in the MDS and ART outcomes: we did not find any significant association with components of MDS (Tables 2S, 3S, 4S, 5S).

DISCUSSION

Principal findings. In our sample of 474 women, adherence to Mediterranean diet, estimated as Mediterranean Diet Score, was not associated to successful IVF outcomes. Moreover, no significant relation was observed between food groups and number of good quality oocytes, availability of embryos for transfer, clinical pregnancy and live birth. We only found a significant slight positive effect of intermediate MDS on good quality oocytes number and achieving clinical pregnancy: however, this did not result in a higher proportion of live birth.

Results. Unlike ours, most published studies have shown a positive relation between healthy diet and successful ART cycles.

In 161 couples undergoing ART treatment in a Fertility Clinic in Rotterdam (The Netherlands) between 2004 and 2007, Vujkovic et al. [20] used a 104-items questionnaire to identify two dietary patterns: a "health-conscious-low processed" pattern, characterized by high intakes of fruits, vegetables, fish, and whole grains and low intakes of processed products (snacks, meats, and mayonnaise), and a Mediterranean one, with high intakes of vegetable oils, vegetables, fish, and legumes and low intakes of snacks. Both patterns were associated with red blood cell folates, but only the Mediterranean diet increased the probability of pregnancy, with an odds ratio of 1.4 (95% CI 1.0-1.9). Neither "health-conscious-low processed" nor the Mediterranean diet were associated with embryo quality.

In a later study, 199 couples were enrolled between 2007 and 2010 in Rotterdam (The Netherlands), if candidate for their first ART cycle [21]. They were asked about their dietary habits using six questions regarding the intake of six main food groups, that is, fruits, vegetables, meat, fish, whole wheat products and fats. Based on this information, an estimate of nutritional habits was calculated. A higher Preconception Dietary Risk score (PDR) indicated a better dietary quality. Clinical pregnancy, that represented the primary outcome, was achieved in 26% of couples. After adjusting for woman's age and smoking habits, partner's PDR, BMI of the couple, and treatment indication, Twigt et al. found that PDR of the woman and the chance of ongoing pregnancy were positively associated (odds ratio 1.6, 95% CI 1.1–2.2).

More recently (2013-2016), in a sample of 244 non-obese women undergoing their first IVF treatment, Karayiannis et al.[9] calculated a Mediterranean diet Score (MedDietScore) ranging between 0 and 55, with higher score indicating greater adherence: in tertiles of MedDietScore, they did not observe a relation with intermediate ART outcomes (oocytes yield, fertilization rate and measures of embryo quality). On the contrary, clinical outcomes such as pregnancy (50% vs 29%) and live birth (49% vs 27%) were significantly higher in the third tertile, as compared to the lower. The association was still present after accounting for age, ovarian hyper-stimulation protocol, BMI, physical activity, anxiety levels, infertility diagnosis, caloric intake and supplements use. Unlike Karayiannis et al.[9], we could not account for supplements, because all women were prescribed folates, and we did not record whether they were taking other supplements on their own initiative.

Recently, in a paper on 357 women who underwent 608 ART cycles in the EARTH study, Gaskins et al. [22] reported that a beneficial effect of Mediterranean diet was observed in women with an intake above the first quartile, but there was no additional benefit with higher adherence to MD, whereas higher adherence to profertility diet showed a linear effect on ART outcomes. In this paper, Gaskins et al. suggested that the Mediterranean diet may be beneficial even at low level of intake, with no additional advantage with increasing adherence. If this finding is true, and considering that in our study MDS was calculated based on the median intakes in this individual population, it is conceivable that even women the lowest score obtained benefit and no difference emerged with higher adherence to MDS. Our study only observed a borderline protective effect of intermediate adherence score to Mediterranean diet on good quality oocyte number and clinical pregnancy in women aged more than 35 years, but no effect on live birth. Excluding alcohol from the score, MDS was associated with good quality embryo number, but this difference was not observed in pregnancy outcomes. At the light of the previous considerations, these findings are not in disagreement with Gaskins' [22]

It has been hypothesized that the beneficial effects of a Mediterranean diet are mainly due to the high intake of vegetable oil [23], containing nutrients such as linoleic acid, and of fruits and vegetables in general, although this association was not observed in a "health-conscious" diet [20] not including the consumption of vegetable oil. On the other hand, it has also been suggested that the intake of high-residue pesticide vegetables may contrast the positive effect of an otherwise healthy diet. It has to be noted, however, that this confounder is unlikely in our study: the 2017 report of

Italian Health Ministry [24] on control of pesticides residues found that less than 1% of horticultural products contained residues over the regulatory limits, whereas 54% did not contain residues at all and 45% was under the regulatory limits.

Inconsistence among studies may be simply due to the fact that diet patterns with the same name are not identical, and may differ enough to impact differently on ART outcomes. Moreover, adherence to a Mediterranean diet is likely higher in Italy than in other countries, and even patients with low MDS may have a higher adherence level to this pattern than individuals in other studies. Thus, the benefit of increasing adherence may not be as dramatic as in other populations.

The inconsistence of our findings with those of Karayiannis et al. [9], despite similarities between considered food groups, may also be due to the fact that our questionnaire did not specify whether cereals (pasta, bread, rice) were consumed as whole or refined. Given that the beneficial effect seemed limited to whole grains intake [25], it is possible that cereals considered in MDS were at least partially refined, thus contributing to increase the glycemic load, associated with higher risk of ovulatory infertility [23]. Indeed, the whole cereals intake in Italy is among the lowest in Europe [26].

Further, the differences observed among our and previous results could be due to different prognostic profile of the considered populations. For example, in our study the overall pregnancy rate was 31.6% vs 42.6% in the study by Karayiannis et al. [9]. Moreover, diet may be act differently in women with different risk profile, in particular it is conceivable that diet may play a major role in couples at best prognosis: whereas in our study female factor was the most frequent reason for couple infertility, infertility was due to female (or combined) factors in less than 10% in Karayiannis et al.[9], in about 26% in Vujkovic et al. [20] and in 36% in Twigt et al. [21]

Clinical implications. Notwithstanding we did not find any association between the adherence to a Mediterranean diet and ART outcomes, at the light of previous research and of general benefits due to a healthy diet (including those on obstetric outcomes), women candidates to ART should be counseled about their dietary habits.

Strengths and limitations. All information was self reported by the woman, so some misclassification could have occurred. However, in Italy, dietary counseling is not routinely advocated by gynecologists before IVF (including in the Center where the study was run), so underreporting of unhealthy diet should be unlikely.

Other sources of bias, including selection or confounding factors, are also unlikely to have produced marked effects, especially considering that all patients were interviewed in the same institution and that participation was practically complete.

With regard to other biases, we analyzed information on nutritional status, and their inclusion into the model did not change the estimated RR. Further, the questionnaire was satisfactorily reproducible [27].

A limitation of this study may be related to some problems of reliability in the measure of physical activity. Indeed, we used a subjective score, with no quantification of total energy expenditure and no validation. However, it has been shown that even simple questions on physical activity may provide useful information. [28]

Another potential limitation is study power. For example, with our data, comparison between the lowest and the highest tertile could identify a risk of pregnancy loss of about 1.8.

Lastly, our findings should be referred only to women of infertile couples and the possibility of unmeasured confounding is always possible.

Conclusions. Our study only observed a borderline protective effect of intermediate adherence score to Mediterranean diet on good quality oocyte number and clinical pregnancy in patients older than 35 years, but no effect on live birth. Overall, our study does not show any linear association between adherence to a Mediterranean diet and oocyte quality or successful IVF.

Authors' contribution

FP and MC designed the research study; MC, BG, and SN contributed to data acquisition and interpretation; FB, ER, SC and FC analyzed the data; ER, ES, MV and FP interpreted the information and wrote the paper.

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Potential conflict of interest.

The authors have no competing interest to declare.

Ethics statement. The study protocol was approved by the Ethical Review Board of Fondazione IRCCS Ca' Granda, Ospedale Maggiore, Policlinico, Milan, Italy (Comitato Etico Milano Area B, reference number 2616, 9 December 2014).

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Table 1. Demographic characteristics of 474 women, according to Mediterranean Diet Score (MDS).

	MDS								P*
	All		0-3		4-5		6-9		
	N	%	N=132	27.8%	N=200	42.2%	N=142	30.0%	
Age (years)									
<35	132	27.8	40	30.3	62	31.0	30	21.1	
35-39	232	49.0	58	43.9	103	51.5	71	50.0	0.14
≥40	110	23.2	34	25.8	35	17.5	41	28.9	
College degree	245	51.7	60	45.4	111	55.5	74	52.1	0.26
Cause of infertility									
Male factor only	124	26.2	36	27.3	57	28.5	31	21.8	
Endometriosis	101	21.3	32	24.2	33	16.5	36	25.4	
Tubal	52	11.0	19	14.4	21	10.5	12	8.4	
Low ovarian reserve	91	19.2	20	15.2	37	18.5	34	24.0	
Ovulatory	20	4.2	5	3.8	8	4.0	7	4.9	0.32
Unexplained	86	18.1	20	15.2	44	22.0	22	15.5	
BMI									
≤24.9	380	80.2	106	80.3	156	78.0	118	83.1	
25.0-29.9	62	13.1	16	12.1	30	15.0	16	11.3	0.32
≥30.0	29	6.1	10	7.6	13	6.5	6	4.2	

On calorie restriction diet in the last year	66	14.1	8	6.1	35	17.8	23	16.6	0.01
Smoking habits									
Never	263	55.5	75	56.8	113	56.5	75	52.8	
Current	86	18.1	32	24.2	31	15.5	23	16.2	0.12
Former	125	26.4	25	18.9	56	28.0	44	31.0	
Occupational physical activity									
Heavy/moderate	131	27.6	40	30.3	56	28.0	35	24.6	
Mainly standing	104	21.9	23	17.4	43	21.5	38	26.8	0.51
Mainly sitting	237	50.0	68	51.5	100	50.0	69	48.6	
Leisure physical activity									
<2 h/wk	255	53.8	79	59.8	110	55.0	66	46.7	
2-4	169	35.6	41	31.1	73	36.5	55	38.7	0.02
≥5	49	10.3	12	9.1	16	8.0	21	14.8	
Previous ART cycle	275	58.0	75	56.8	111	55.5	89	62.7	0.28
Previous ovarian surgery	65	13.7	18	13.6	24	12.0	23	16.2	0.52
Type of ART procedure (n=451)									
IVF	177	39.2	47	37.0	70	36.1	60	46.2	0.13
ICSI	274	60.8	80	63.0	124	63.9	70	53.8	
	Mean or median	**SD or IQR**	**Mean or median**	**SD or IQR**	**Mean or median**	**SD or IQR**	**Mean or median**	**SD or IQR**	**P**
Mean calories, Kcal/day	1749	446	1624	358	1752	480	1860	441	<0.0001
FSH, mUI/mL	7.3	5.8-8.9	7.2	5.7-8.7	7.3	5.7-8.6	7.3	5.9-9.6	0.65

AMH, ng/mL	1.6	0.8-3.2	1.7	0.8-3.0	1.9	0.9-3.5	1.4	0.8-2.8	0.14
Good quality oocytes	5.0	3.0-8.0	4.0	3.0-7.0	6.0	3.0-8.0	4.0	2.0-7.0	0.053
Good quality embryos (n=451)	2.0	1.0-3.0	1.0	0.0-3.0	2.0	1.0-3.0	2.0	1.0-4.0	0.26
Successful outcomes	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**P**
Embryo transfer	414	87.3	114	86.4	178	89.0	122	85.9	0.89
Clinical pregnancy	150	31.6	37	28.0	73	36.5	40	28.4	0.98
Live birth	117	24.7	33	25.0	51	25.5	33	23.4	0.73

Sometimes the sums do not add up to the total because of missing values.

* Cochrane-Mantel-Haenszel chi-square

BMI: body mass index; ART: assisted re production techniques; SD: standard deviation; IQR: interquartile range; FSH: follicle-stimulating hormone; AMH: anti-mullerian hormone

Table 2. Relative risks for failure in clinical outcomes of ART, in 474 women according to Mediterranean Diet Score.

		Number of good quality oocytes		**Number of good quality embryos**		**Embryo transfer**		**ARR (95% CI)**	**Clinical pregnancy**		**ARR (95% CI)**	**Live birth**		**ARR (95% CI)**
MDS		median	95% CI	median	95% CI	Yes	%		Yes	%		Yes	%	
Overall														
0-3	132	4.8	3.9-5.8	1.4	0.9-2.0	114	86.4	1	37	28.0	1	33	25.0	1
4-5	200	5.4	4.4-6.3	1.7	1.2-2.3	178	89.0	0.83 (0.46-1.50)	74	37.0	0.95 (0.86-1.05)	51	25.5	1.00 (0.90-1.11)
6-9	142	4.6	3.7-5.6	1.9	1.3-2.5	122	85.9	0.86 (0.47-1.55)	40	28.2	0.98 (0.87-1.09)	33	23.2	0.99 (0.89-1.11)
P for trend			p=0.64		p=0.09			p=0.64			p=0.68			p=0.87
≤35 years old														
0-3	51	5.9	4.5-7.4	1.5	0.8-2.4	48	94.1	n.e.	20	39.2	1	19	37.2	1
4-5	79	6.2	4.8-7.8	1.9	1.1-2.9	72	91.1	n.e.	34	43.0	0.96 (0.80-1.14)	27	34.2	1.00 (0.81-1.21)
6-9	44	5.1	3.7-6.7	2.1	1.2-3.3	40	90.9	n.e.	17	38.6	0.99 (0.81-1.20)	15	34.1	1.00 (0.79-1.26)
Trend			p=0.32		p=0.17						p=0.85			p=0.98
>35 years old														
0-3	81	4.3	3.1-5.6	1.4	0.8-2.1	66	81.5	1	17	21.0	1	14	17.3	1
4-5	121	5.1	3.9-6.4	1.6	1.0-2.3	106	87.6	0.70 (0.36-1.36)	40	33.1	0.84 (0.70-1.00)	24	19.8	0.96 (0.84-1.10)
6-9	98	4.3	3.1-5.5	1.6	1.0-2.4	82	83.7	0.79	23	23.5	0.94	18	18.4	0.97

								(0.41-1.52)			(0.78-1.13)			(0.84-1.12)
Trend			p=0.87		p=0.42			p=0.52			p=0.50			p=0.73

ARR: adjusted relative risk; CI: confidence interval

The final model included age class (<35, 35-39, ≥40, when appropriate), previous ART cycles (no, yes), leisure physical activity (<2, 2-4, ≥5 hours/week), smoking (never, current, former), obesity (BMI<30.00, ≥30.00), daily calories intake (Kcal, continuous variable), calorie restriction diet in the last year (no, yes).

n.e.: estimates were not evaluable in the full model, due to the low number of embryo transfer failures in the reference category.