Men's Dietary Patterns in Relation to Treatment Outcomes among Couples Undergoing Infertility Treatment with Assisted Reproductive Technology

MEN'S DIETARY PATTERNS IN RELATION TO TREATMENT OUTCOMES AMONG COUPLES UNDERGOING INFERTILITY TREATMENT WITH ASSISTED REPRODUCTIVE TECHNOLOGY

by

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Thank you,

Makiko Mitsunami

OVERVIEW OF THE THESIS PAPERS

Infertility is a major health concern worldwide, especially in societies where delayed childbearing is becoming more common. While much of the focus on understanding the causes of infertility focuses on women, male factors account for approximately half of the infertility burden (1). Standard semen analysis is not only an important biomarker of spermatogenesis and testicular function but is also the cornerstone for clinical diagnosis of male factor infertility (1,2). However, it is known that semen analysis is not a perfect predictor of a couple's fertility, both in couples attempting conception on their own and in couples attempting conception with medical assistance (3,4). Men's diet has been reported as a potentially modifiable factor influencing semen quality (Arrow A in Figure 1 of Overview)(5–8). However, there is little data evaluating the impact of men's diet on a couple's fertility (Arrow C in Figure 1 of Overview) (9–11). There is a particularly important knowledge gap as some data suggests that associations between diet and semen quality do not necessarily translate into associations with couple-based outcomes, such as fertility (Arrow B in Figure 1 of Overview) (9–14).

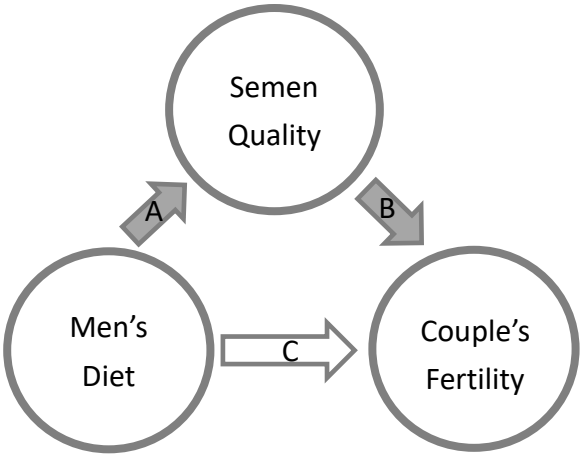
Traditionally, research aimed at understanding the role of nutrition on health has focused on trying to identify the impact of individual foods or nutrients. However, this approach has important shortcomings as it fails to account for known and unknown complex

interactions between different nutrients, foods, and non-nutritive components of food. Moreover, results from research focused on individual foods or nutrients are not always easy to translate into clinical or public health recommendations as foods and nutrients are not eaten in isolation but are rather part of dietary patterns. Acknowledging this reality, in this thesis, I have decided to focus on understanding the role of men's dietary patterns on semen quality and infertility treatment outcomes. In general terms, diet patterns can be defined by either an *a priori* (hypothesis-oriented) approach which generally involves the calculation of diet scores based on a fixed set of external criteria (15) or an *a posteriori* (data-driven) whereby patterns are identified based on correlation patterns between individual foods/nutrients, correlation patterns between diet and intermediate biomarkers, correlation patterns between different individuals, or a combination thereof (16–18). While these different approaches have strengths and limitations, a strength of data-driven approaches is that these can generally account for the totality of diet (rather than specific aspects of diet) and by relying solely on data, any resulting diet pattern is not influenced by pre-existing knowledge or beliefs of what the relation between diet and health outcomes should be. Therefore, we investigated the impact of men's diet on couple's infertility treatment outcomes using prospective observational study data using two different data-driven to identify dietary patterns.

Paper 1: We identified underlying dietary patterns among men using principal component analysis (PCA) and then investigated the association between adherence to the resulting dietary patterns and outcomes of infertility treatment with assisted reproductive technology (ART). This analysis provides insights into how the impact that actual dietary behavior observed in men presenting to fertility centers may affect ART outcomes, regardless of the underlying biological mechanisms linking these.

Paper 2: We empirically derived a dietary score capturing the overall association of diet with semen quality using reduced rank regression (RRR) and then examined this score in relation to outcomes of infertility treatment with ART. This analysis provides insights into how dietary factors influencing semen quality parameters may in turn impact ART outcomes.

FIGURE 1 OF OVERVIEW. Directed acyclic graph of the association between men's diet and a couple's fertility.*



* We approached both white and grey arrows in paper1.

Grey arrows indicate path which was mediated by semen quality we approached in paper2.

PAPER 1 (Submitted to Journal of Assisted Reproduction and Genetics, status: revision)

Men's dietary patterns in relation to infertility treatment outcomes among couples undergoing in vitro fertilization

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Abstract

Purpose(s): To evaluate the relationship of men's dietary patterns with outcomes of *in vitro* fertilization (IVF).

Methods: This is a prospective cohort study including 231 couples with 407 IVF cycles, presented at an academic fertility center from April 2007 to April 2018. We assessed diet with a validated food frequency questionnaire and identified Dietary Pattern One and Dietary Pattern Two using principal component analysis. We evaluated adjusted probability of IVF outcomes across the quartiles of the adherence to two dietary patterns by generalized linear mixed models.

Results: Men had a median age of 36.8 years and BMI of 26.9 kg/m^2. Women's median age and BMI were 35.0 years and 23.1 kg/m^2, respectively. Adherence to Dietary Pattern One ($r_{Pearson}$=0.44) and Dietary Pattern Two ($r_{Pearson}$=0.54) was positively correlated within couples. Adherence to Dietary Pattern One was positively associated with sperm concentration. A 1-unit increase in this pattern was associated with a 13.33 (0.71-25.96) million/mL higher sperm concentration. However, neither Dietary Pattern One nor Dietary Pattern Two were associated with fertilization, implantation, clinical pregnancy, or live birth probabilities.

Conclusions: Data-derived dietary patterns are associated to semen quality but unrelated to

the probability of successful IVF outcomes.

Keywords: Dietary pattern, male subfertility, in vitro fertilization, probability of live birth

Declarations:

Funding

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Conflicts of interest/Competing interests

Not aplicable for Makiko Mitsunami, Albert Salas-Huetos, Lidia Mínguez-Alarcón, Jill A. Attaman, Jennifer B. Ford, Martin Kathrins, Irene Souter.

Jorge E. Chavarro received a grant from the National Institutes of Health.

Availability of data and material

Data are available upon request after approval of data sharing and use agreements between institutions.

Code availability

All analyses were performed using SAS university edition with VirtualBox version 6.1.6.

Code is available upon request after approval of data sharing and use agreements between institutions.

Authors' contributions

Jorge E. Chavarro was involved in study concept and design, critical revision for important intellectual content of the manuscript, and had a primary responsibility for final content. Makiko Mitsunami drafted the manuscript, analyzed data; Albert Salas-Huetos reviewed the statistical analysis; Lidia Mínguez-Alarcón contributed to providing the data; Jennifer B. Ford and Irene Souter were involved in acquisition of the data; Makiko Mitsunami, Albert Salas-Huetos, Lidia Mínguez-Alarcón, Jill A. Attaman, Jennifer B. Ford, Martin Kathrins, Irene Souter., and Jorge E. Chavarro interpreted the data; All authors were involved in the critical revision of the manuscript and approved the final manuscript.

Ethics approval

The EARTH study was approved by the Institutional Review Board of both MGH and the Harvard T.H. Chan School of Public Health.

Consent to participate

All participants to the EARTH study completed written consent forms.

Consent for publication

The written consent forms included the permission for publication.

Introduction:

Infertility, defined as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse, is a worldwide issue (1) affecting 15% of reproductive age couples (2). Male factor is one of the most common causes of infertility and male infertility evaluation is important not only for defining infertility treatment strategies but also for men's health itself as male infertility could be a predictor of future morbidity (3). Contributing factors to male infertility are various; genetic factors, environmental factors such as smoking, alcohol consumption, psychological stress, substance abuse, exercise, and comorbidities including cardiovascular disease, hyperlipidemia, diabetes, and obesity (4–10).

Previous epidemiological work suggests that paternal dietary patterns associated with lower risk of cardiovascular disease and other chronic conditions (11–13), such as the Mediterranean diet and the dietary approaches to stop hypertension (DASH) diet may be positively associated with semen quality. On the other hand, dietary patterns favoring intakes of red and processed meats, animal fat, refined grains, and sweets – which have been related to a higher risk of non-communicable chronic diseases (14) – may affect negatively semen quality (15–20). However, semen parameters are not perfectly correlated with a couple's fertility (21,22). Moreover, while some evidence suggests men's diet may influence a couple's

fertility (23–25), data on the extent to which men's diet could impact fertility in couples trying to conceive naturally or with medical assistance remains scarce. To address this important question, we evaluated the association between adherence to men's dietary patterns identified in the study population using a data-driven approach and outcomes of infertility treatment with *in vitro* fertilization (IVF). We hypothesized that couples with a male partner with greater adherence to dietary patterns associated with lower risk of cardiovascular disease and other chronic comorbidities would have higher clinical pregnancy and live birth rates during the course of infertility treatment with IVF.

Materials and Methods:

Study population

Couples presenting for infertility evaluation and treatment to the Massachusetts General Hospital (MGH) Fertility Center were invited to enroll in the Environment and Reproductive Health (EARTH) Study. Established in 2004, this cohort study investigates the effect of environmental and dietary factors on fertility and pregnancy outcomes; study design has been described in detail elsewhere (26). Men (ages 18-55) and women (ages 18-45) completed several study questionnaires which included demographics, medical, reproductive, and occupational history, and lifestyle, and underwent an anthropometric

evaluation after providing written consent. Diet assessments were introduced in April 2007. Couples were encouraged but not required to join as a couple. This study included all couples where the male partner completed a food frequency questionnaire (FFQ) and his female partner started at least one IVF cycle by April 2018. There were 377 male partners who joined the study from April 2007 to April 2018. Of these, 146 men did not complete diet assessments leaving 231 men eligible for the current study. Baseline demographic and reproductive data of participants included in the study did not differ substantially from those excluded (Supplemental Table 1). The study was approved by the Institutional Review Board of both MGH and the Harvard T.H. Chan School of Public Health.

Dietary Assessment and identification of dietary patterns

Diet was assessed using an extensively validated FFQ (27,28). Participants were asked to report how often, on average, they consumed each of the 131 foods and beverages in the questionnaire during the previous year, with frequency choices ranging from 'never or less than once per month' to 'six or more times per day.' Individual food items were grouped into 42 pre-defined food groups based on those proposed by Hu and colleagues (29). We used these 42 food groups to identify underlying dietary patterns in the study population using Principal Component Analysis (PCA) with Varimax rotation in order to achieve a simpler and

more interpretable structure. The number of factors retained was determined based on Eigenvalues, the scree plot and interpretability of the resulting factors.

Clinical outcomes

The primary outcome of this study was the probability of live birth per initiated treatment cycle. Live birth was defined as the birth of a neonate at or after 24 weeks of gestation. Secondary outcomes were fertilization rate, the probability of implantation, and clinical pregnancy. Fertilization rate was defined as the number of two pronuclei embryos divided by the number of metaphase II oocytes and evaluated by mode of insemination (Conventional insemination or intracytoplasmic sperm injection (ICSI)). Successful implantation was defined as an elevation of serum β-hCG level greater than 6 mIU/mL measured at approximately 2 weeks after embryo transfer. Clinical pregnancy was defined as the presence of an intrauterine gestational sac on ultrasound at 6 gestational weeks.

Semen parameters assessment

Secondary outcomes included conventional semen parameters (ejaculate volume, total sperm count, sperm concentration, total motility, progressive motility, and the percentage of sperm normal morphology). We used data from semen samples collected for diagnostic

purposes as well as pre-processing data for samples collected for treatment purposes. Men provided semen specimens on-site via masturbation and completed questionnaires of their abstinence time. A 48 hour abstinence time before sample collection was recommended. For this study, we included all semen sample data even if the abstinence time was not adhered to. Semen parameters were assessed based on the 2010 WHO manual guideline (30). Semen samples were inspected after 30-mins liquefication on a 37 °C incubator. Ejaculate volume was calculated from sample weight, assuming a semen density of 1g/ml. Sperm concentration and motility were assessed by computer-assisted semen analysis (CASA; 10HTM-IVOS, Hamilton-Thorne Research, Beverly, MA) (31). Motile spermatozoa were evaluated as total motility (progressive motility + non-progressive motility), progressive motility, non-progressive motility, and immotile sperm (30). Total sperm count (million/ejaculate) referred to total number of spermatozoa in the entire ejaculate which was calculated by multiplying sperm concentration by ejaculated volume. Sperm morphology (% normal) was assessed on two slides per specimen (with a minimum of 200 cells assessed per slide) via a microscope with an oil-immersion ×100 magnification (Nikon, Tokyo, Japan). Then, men was dichotomized as having normal or below normal morphology according to Strict Kruger scoring criteria (32).

Statistical Analysis

Men were categorized into quartiles according to adherence to PCA-derived dietary patterns. We first examined differences in demographic, nutritional and reproductive characteristics, and semen parameters by quartile of adherence to dietary patterns using the Kruskal-Wallis test for continuous variables and Fisher exact test for categorical variables. Chi-square test were used for evaluating differences across categories of primary infertility diagnosis and initial stimulation protocol as the Fisher's test did not run with these two variables. There were a total 20 couples (9.4%), 33 missing values (8.1%) in age, BMI, education level, race, smoking status. For imputation, the median values were assigned for age and BMI and the most frequent category were assigned for race, smoking status, and education levels. For dietary assessment, 18 women (8.5%), 40 cycles (9.8%) had missing data, but they were not imputed. We investigated the relationship between two PCA-derived patterns and semen quality using linear mixed models with random intercepts, to account for multiple IVF cycles per couple, adjusting men's age, total calorie intake, body mass index (BMI), race, smoking status, education level, and physical activity. The association of adherence to PCA-derived dietary patterns with IVF outcomes was examined using generalized linear mixed models with random intercepts, to account for multiple IVF cycles per couple, while adjusting for potential confounders. We used population marginal means to present results as

probabilities and 95% confidence intervals (95% CI) adjusted for the covariates at their average levels for continuous variables and weighted average level of categorical variable in the model (33). Tests for linear trend were conducted by modeling quartiles of adherence as a continuous variable. Confounding was evaluated using prior scientific knowledge and differences in baseline patient characteristics by dietary pattern adherence. The initial multivariable-adjusted model included terms for men's age and total calorie intake. The second model included additional terms for men's body mass index (BMI), race, smoking status, education level, and physical activity, as well as women's age and BMI, the couples' primary infertility diagnosis, treatment protocol and indicators for missing covariate data. The third model included all variables of the second model and terms for women's adherence to the two dietary patterns, race and smoking status.

Sensitivity analyses were performed to evaluate the robustness of the findings. These included restricting all analyses to couples with complete female diet data, and to the first treatment cycle for each couple for IVF outcomes. We also conducted analyses stratified by primary infertility diagnosis (male factor vs. female and unexplained factor), IVF treatment history, and past pregnancy history. In addition, we conducted stratified analysis of previous infertility examination to detect association between dietary pattern and semen quality. All analyses were performed using SAS university edition with VirtualBox version 6.1.6.

Results:

A total of 231 couples who underwent 407 IVF cycles were included in the analysis. Median age of male partners at enrollment was 36.8 years (interquartile range (IQR): 33.4-40.0 years) and median BMI was 26.9 kg/m^2 (IQR: 24.1-29.1 kg/m^2). Most men were white (89.2%) and had never smoked (66.2%) (Table 1). Women had a median baseline age and BMI of 35.0 years (IQR: 32.0-38.0 years) and 23.1 kg/m^2 (IQR: 21.0-25.7 kg/m^2), respectively. Male factor infertility was the most common initial primary infertility diagnosis (36.8%). Two dietary patterns were identified using PCA (Figure 1, Supplemental Table 2 and Supplemental Figure 1). The first pattern, labeled as Dietary Pattern One, was characterized by greater intakes of processed meats, unprocessed red meats, high fat dairy, beer, french fries, cream soups, refined grains, pizza, snacks, and sweets. The second pattern, labeled as Dietary Pattern Two, was characterized by greater intakes of fruit, vegetables, legumes, soy foods, whole grains, nuts and nut butters. Intakes of organ meats, fish, chicken, eggs, margarine, low-fat dairy, liquor, wine, tea, coffee, fruit juice, cold breakfast cereal, salad dressings, artificial sweeteners, and water did not have high loading scores on either of the identified dietary patterns.

Men's adherence to Dietary Pattern One was associated with higher BMI. Men's adherence to Dietary Pattern Two was inversely related to BMI and positively related to educational

attainment. Adherence to both patterns was positively related to higher total calorie intake. Moreover, adherence to Dietary Pattern One ($r_{Pearson}$=0.44) and Dietary Pattern Two ($r_{Pearson}$=0.54) were positively correlated within couples (Table 1). Supplemental Table 3 shows the distribution of semen quality parameters among male participants. Approximately 40-60% of participants demonstrated asthenospermia according to the WHO reference limits (30).Adherence to Dietary Pattern One was positively related to sperm concentration. A 1-unit increase in this pattern was associated with a 13.33 (0.71-25.96) million/mL higher sperm concentration (Table 2). This association was stronger among men who had undertaken an infertility examination prior to joining the study (β=17.93 (3.55 to 32.30) million/mL). The association was in the opposite direction among men who had not undertaken an infertility examination before joining the study, although sample size was limited in this group (Table 2).

There was no association between men's adherence to two data-derived dietary patterns and fertilization rate in total cycles, stratified analyses for IVF cycles using conventional insemination and ICSI cycles (Figure 2). There was also no discernible association of men's adherence to either dietary pattern with probabilities of implantation, clinical pregnancy or live birth in multivariable-adjusted models without (Figure 3) or with adjustment for women's adherence to the same dietary patterns (Figure 4).

In sensitivity analyses, results were consistent with the primary findings when analyses were restricted to couples with complete female diet data (Supplemental Figure 2) and to the first treatment cycle for each couple (Supplemental Figure 3). Similarly, analyses stratified by a primary infertility diagnosis (Supplemental Figure 4), past IVF treatment history (Supplemental Figure 5), or past pregnancy history (Supplemental Figure 6) showed no association between the dietary patterns and IVF outcomes either.

Discussion:

We investigated the association of men's adherence to two data-derived dietary patterns with semen quality and outcomes of infertility treatment with IVF. Despite sperm concentration being associated with one of these patterns, we found no evidence that men's adherence to these dietary patterns had any meaningful impact on the outcome of infertility treatment with IVF. To our knowledge, this is the first study to date examining the association between men's dietary patterns and couples' IVF outcomes. Hence, it is important that this question is revisited in additional studies.

The finding that higher adherence to Dietary Pattern One was associated with higher sperm concentration stands in contrast with several observational studies suggesting that male adherence to dietary patterns linked to higher chronic disease risk could also have a

negative impact on semen quality (17,34,35), whereas the opposite appears to be the case for adherence to diet patterns previously related to lower risk of chronic conditions (15–18,34–36). A possible reason for the observed relation and the inconsistency with previous literature is that prior knowledge of results of semen analyses, and possibly other diagnostic information, may change the way in which men eat or engage in other behaviors with the goal of optimizing their fertility. For example, if men who know that their semen analysis was above the WHO reference limit do not change their diet but men who know that there are abnormal results in their semen analysis decide to change their diet in response to this information, we would expect that any association between diet and semen quality would be more reflective of this differential change in behavior than of any influence that diet may have on semen quality. This situation is analogous to what has been previously described for paradoxical associations in cross-sectional studies of diet with outcomes that are known to participants, such as the cross-sectional association between intake of diet sodas and BMI (37,38). Our findings, and in particular the divergent pattern after stratification by whether or not men had undergone diagnostic procedures prior to joining the study, are in line with this interpretation and highlights a potential peril of conducting research on behavioral determinants of semen quality in clinical populations.

Data on men's diet and a couple's fertility is scant, yet the literature on diet and semen

quality has been interpreted as implying positive effects on couple fertility. However, the evidence base to support this inference is weak. To start, semen quality is known to be a weak predictor of probability of conception both among couples attempting conception on their own as well as in couples trying to conceive with medical assistance (21,22). Moreover, in previous reports from this cohort we have found that specific dietary factors related to semen quality, such as intake of processed meat, dairy and soy foods (39–41), are unrelated to infertility treatment outcomes (23,42,43). Conversely, we have found that dietary factors that have been consistently found to be unrelated to semen quality, such as intakes of alcohol and caffeine (5,6), were, paradoxically, related to live birth rates during the course of IVF (44). This does not mean that there are not specific nutritional factors that can impact couple fertility by improving semen quality. For example, we and others have documented positive associations between intake of fish, fish oil, or marine fatty acids with better semen quality and other markers of testicular function (45–48) and, independently, with greater fecundability (24). Nevertheless, the discrepancies highlight the fact that improvements in semen quality do not necessarily imply improvements in IVF outcomes for couples undergoing treatment and that, therefore, the identification of male partner characteristics that impact a couple's fertility requires the direct evaluation of IVF outcomes as the study outcome rather than relying on semen quality as a proxy outcome as has been traditional in

andrology and reproductive medicine.

It is important to mention that male effects on reproduction not mediated through traditional semen quality parameters are not purely theoretical. Although the evidence base is still emerging, there is literature showing that the sperm genome and epigenome may play an important role in fertility (49,50) and are subject to environmental modification (51,52). Also, while not directly examining fertility, emerging experimental data suggests that paternal environmental exposures could exert effects on pregnancy and offspring health through the sperm epigenome (53–55). For example, folate-deficient, high-fat, or low-protein diets in males, but not females, negatively impact offspring's metabolism through epigenetic mechanisms (53,55). These findings suggest that men's diet can impact reproduction through additional mechanisms.

As discussed above, a possible interpretation of our findings is that, despite previous research relating dietary patterns to semen quality, men's diet has no impact on a couple's outcomes through IVF. The interpretation of a lack of effect is also in line with findings from two recent randomized trials which found no effect on live birth rate of supplementing men in infertile couples with custom micronutrient formulations (56,57). A related explanation for the lack of association is the fact that we studied this question among couples undergoing infertility treatment with IVF. ICSI has been the most widely utilized assisted reproductive

technology over the recent years (58) and IVF/ICSI is a powerful intervention for male factor infertility and impaired semen quality (primarily on concentration and motility) that may completely offset comparatively smaller impacts of men's diet on semen quality. If this is the case, reexamining this question among couples attempting conception without medical intervention is essential. It is also important to consider alternate interpretations. One possibility is that the data-derived dietary patterns did not capture food groups that could impact fertility. For example, fish intake was not part of either of the dietary patterns identified in this analysis, but has been previously linked to a couples' fertility (24). Clearly, additional work aimed at identifying male partner factors, including modifiable lifestyle factors, that could impact a couple's fertility is necessary.

It is also important to interpret the results in light of the study's limitations and strengths. First, our study had only a modest number of participants and therefore our statistical power to detect small to modest associations was limited. Second, we only assessed diet at baseline FFQ and hence we were unable to document any changes in diet over time as couples underwent treatment. This could result in a dilution of the actual relationship, particularly for couples who take longer to conceive, either due to longer intervals from enrollment to first treatment cycle or more failed treatment cycles, or are never able to conceive. However, sensitivity analysis restricted to each couple's first cycle were consistent with the primary

findings. Also, this study was conducted at an academic fertility center and more than 80% of the men had already been examined before the study baseline. Therefore, it is important to consider the extent to which associations with semen quality reflect an effect of diet on spermatogenesis or the effect of being aware of one's semen quality on subsequent dietary behaviors. Third, although we used an extensively validated diet questionnaire, measurement error is still unavoidable in questionnaire-based studies of diet. Given that diet assessment preceded treatment outcome assessment the expected effect would be an attenuation of effect estimates towards the null. Fourth, the loading factor was relatively low, especially in comparison to other applications of PCA, such as development of psychometric tools and dimension reduction of highly correlated high-dimensional data such as used in Genome-Wide Association Studies data to account for population stratification. However, the loading factors identified here are not too different from those reported in previous work using PCA to identify dietary patterns specifically (17,59). However, the same technique has been used extensively in nutritional epidemiology to identify dietary patterns and associations with a variety of health outcomes (60–62). Thus, this is unlikely to be the reason why we failed to observe an association with our primary outcome. In addition, as mentioned above, investigating the role of men's diet on fertility among couples undergoing IVF could completely obscure a true but modest effect of men's diet on couple fertility and therefore

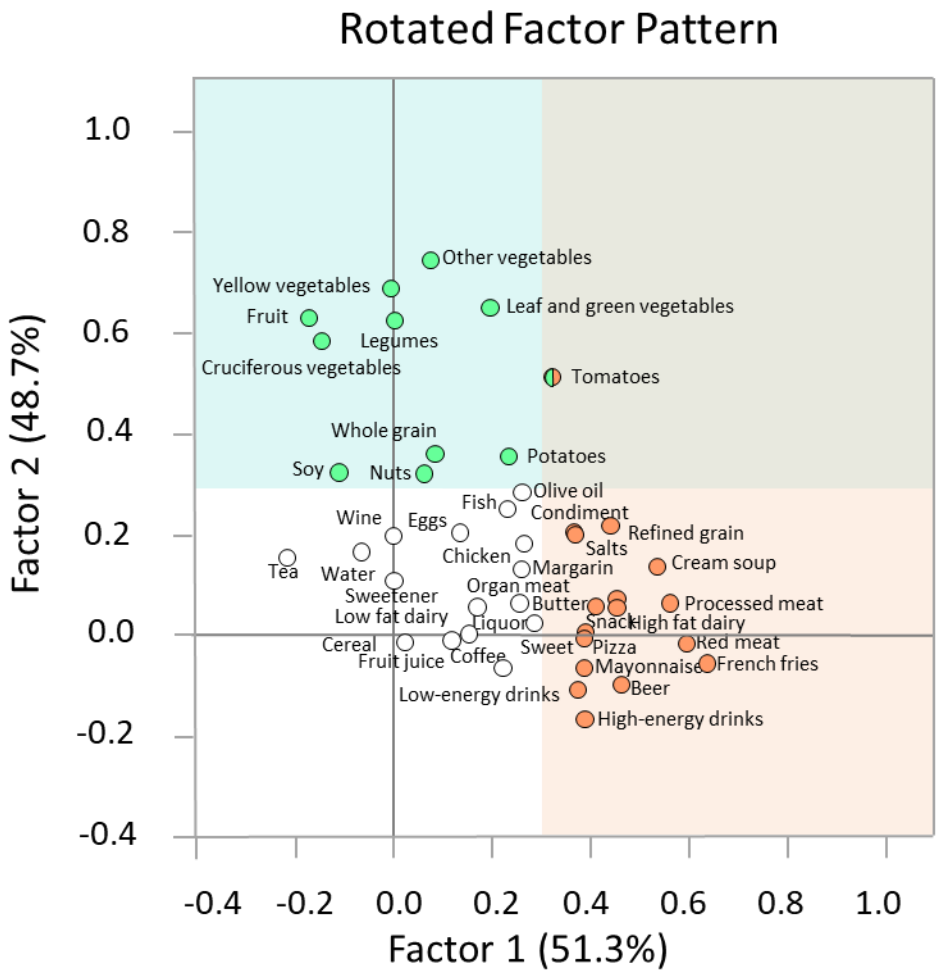
results cannot be generalized to couples attempting conception naturally. Last, the study population was primarily white. While this certainly limits generalizability to other racial groups, the race distribution in our study closely mirrors that of couples undergoing infertility treatment nationwide and therefore results can still inform practice. Strengths of the study include the recruitment of both male and female partners, which is, unfortunately, still not the norm in fertility studies and allowed us to inquire about the role men's diet may have on fertility. Moreover, it allowed us to take into consideration within-couple correlations in diet and other relevant behavioral and demographic factors that could influence the outcome of infertility treatment. The study's prospective design with complete follow-up of clinically relevant outcomes including live birth rate, the extensive collection of key lifestyle factors in both partners and the use of validated instruments also add to the study's strengths.

In brief, results from this study suggest that men's adherence to two data-derived dietary patterns was not related to outcomes of infertility treatment with IVF in spite of an association between Dietary Pattern One and sperm concentration. These results differ with the expanding literature suggesting that adherence to healthy dietary patterns is related to better semen quality. Given the scarcity of data on this topic, it is important that additional studies examine the role of men's diet on fertility both in the setting of infertility treatment

and among couples attempting conception without medical assistance.

Figures:

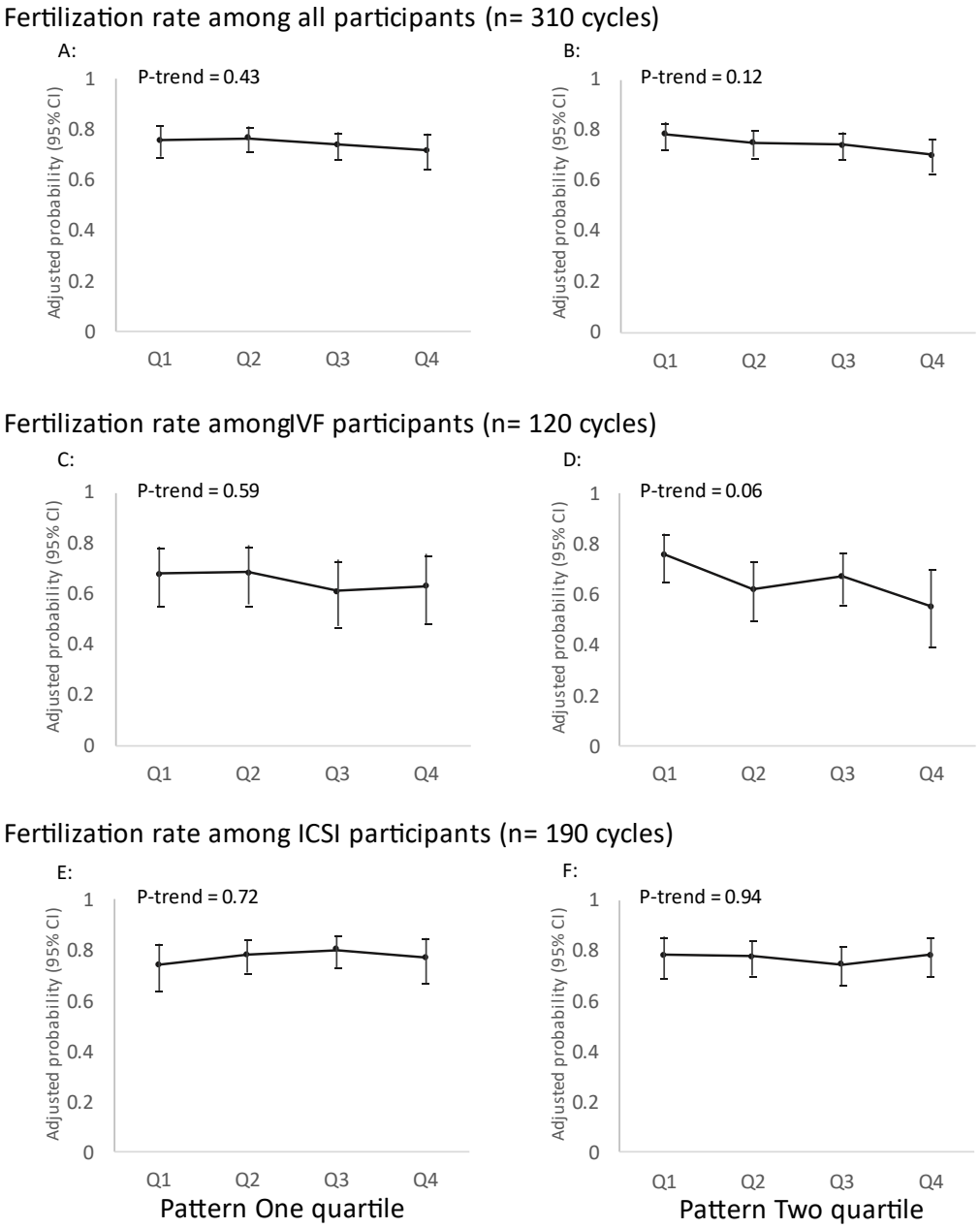
Figure 1. Principal component analysis plot with two factor loadings for food groups among 231 couples undergoing infertility treatment. *



* Orange: Food groups which had Factor 1 loading greater than 0.3.

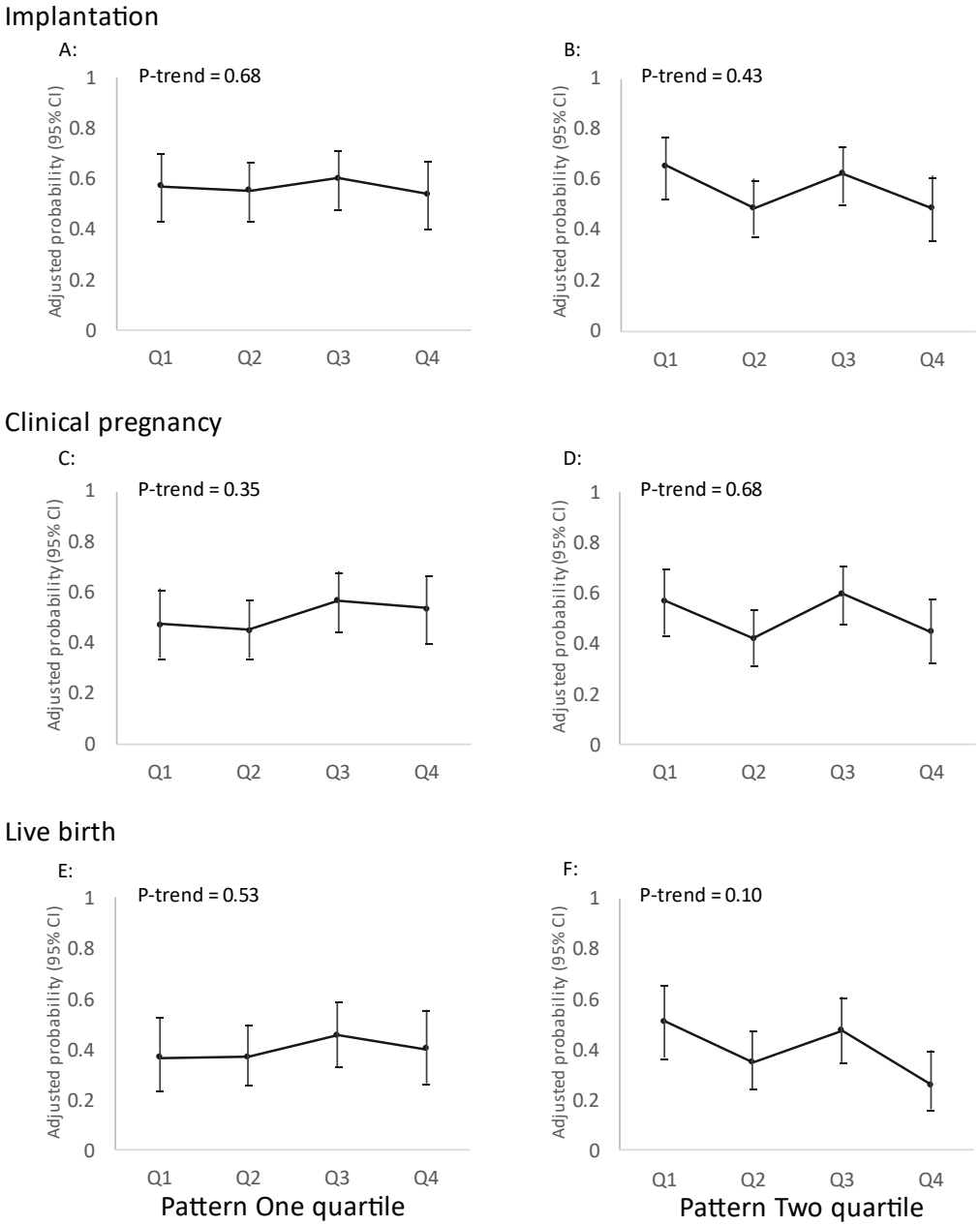
Turquoise: Food groups which had Factor 2 loading greater than 0.3.

Figure 2. Men's adherence to Dietary Pattern One and Two in relation to fertilization rate.*



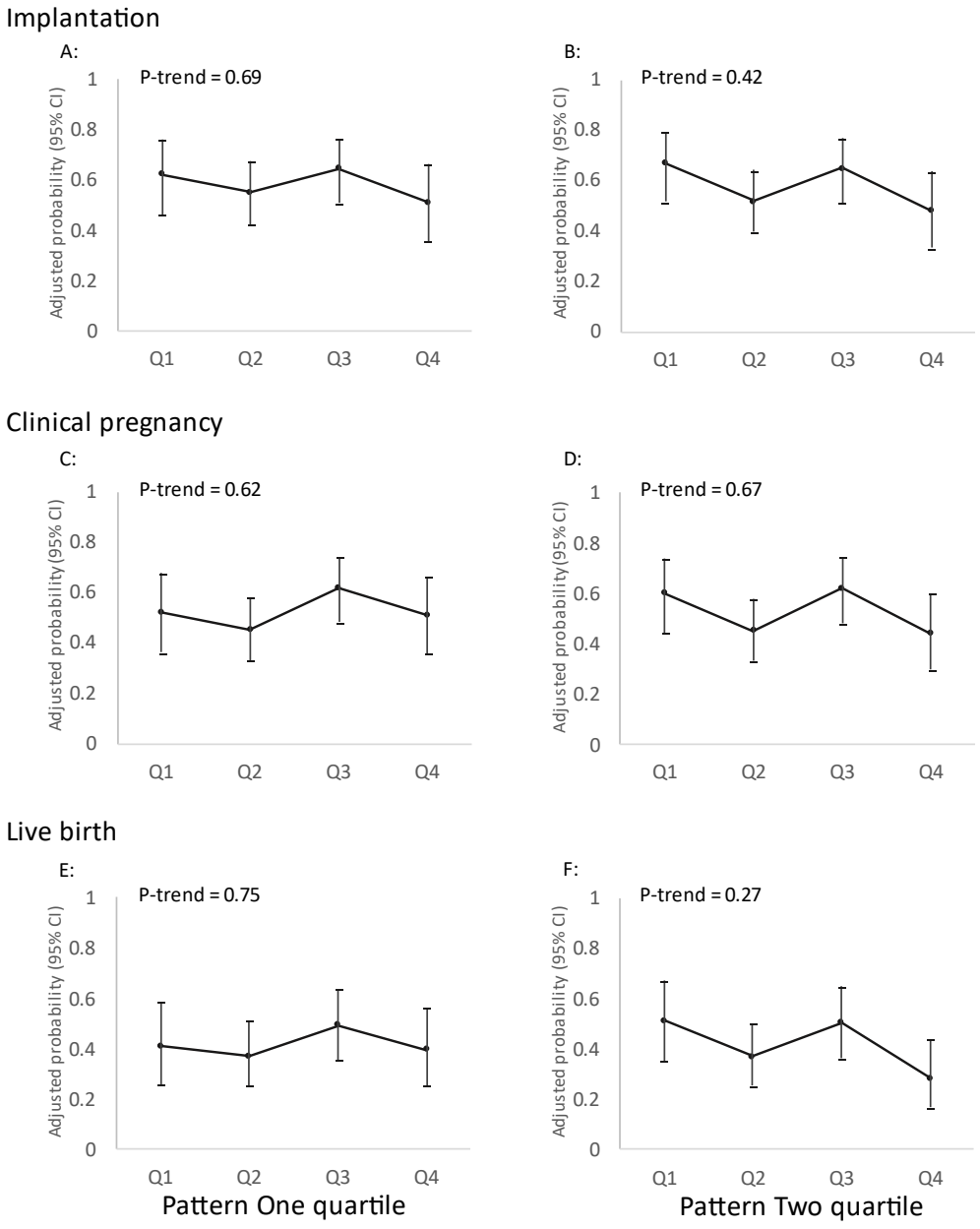
* Adjusted for men's age, total calorie intake, BMI, race, smoking status, education level, and moderate-to-vigorous physical activity; women's age and BMI; primary infertility and treatment protocol. Q: quartile.

Figure 3. Men's adherence to Dietary Pattern One and Two in relation to clinical outcomes of infertility treatment with IVF (N = 231 couples, 407 cycles).*



* Adjusted for men's age, total calorie intake, BMI, race, smoking status, education level, and moderate-to-vigorous physical activity; women's age and BMI; primary infertility diagnosis and treatment protocol.

Figure 4. Men's adherence to Dietary Pattern One and Two in relation to clinical outcomes of infertility treatment with IVF after co-adjustment for women's adherence to the same diet patterns (N = 213 couples, 367 cycles).*



* Adjusted for men's age, total calorie intake, BMI, race, smoking status, education level, and moderate-to-vigorous physical activity; women's age, BMI, and adherence to dietary pattern one and two; primary infertility diagnosis and treatment protocol. Q: quartile.

Tables:

Table 1. Baseline demographic, nutritional and reproductive characteristics of study participants, overall and in lowest and highest quartiles of adherence to Dietary Pattern One and Two.*

	Total	Dietary Pattern One				
		Q1	Q2	Q3	Q4	P**
n	231	57	58	58	58	
Demographics characteristics						
Age(y)	36.8(33.4-40.0)	37.9(34.1-40.0)	36.3(32.9-39.2)	36.6(33.0-40.6)	36.0(33.6-40.5)	0.67
BMI(kg/m^2)	26.9(24.1-29.1)	26.6(23.7-29.3)	25.4(23.7-27.4)	27.3(25.8-30.0)	27.5(25.4-29.4)	0.01
Race, white	206(89.2)	47(82.5)	52(89.7)	52(89.7)	55(94.8)	0.21
Smoking, never smoker	153(66.2)	44(77.2)	38(65.5)	37(63.8)	34(58.6)	0.19
Education, college or higher	183(84.7)	48(90.6)	44(84.6)	45(80.4)	46(83.6)	0.51
Moderate-to-strenuous exercise(min/week)	165 (60 390)	150(60-330)	169(59-432)	253(90-450)	168(47-390)	0.28
Calories(kcal/day)	1934(1586 2384)	1530(1225-1843)	1785(1483-2096)	2025(1700-2258)	2585(2165-2934)	<0.001
Reproductive characteristics						
History of varicocele	19(8.2)	4(7.0)	5(8.6)	6(10.3)	4(6.9)	0.95
Female partner characteristics						
Age(y)	35.0(32.0-38.0)	35.0(32.0-39.0)	35.0(32.0-37.0)	35.0(33.0-38.0)	35.0(32.0-38.0)	0.78
BMI(kg/m^2)	23.1(21.0-25.7)	22.0(20.5-25.5)	22.8(20.8-24.5)	23.7(21.4-25.9)	23.9(21.6-27.9)	0.11
Race, white	194(84.4)	40(70.2)	53(93.0)	52(89.7)	49(84.5)	0.0064
Smoking, never smoker	166(72.2)	41(71.9)	46(80.7)	36(62.1)	43(74.1)	0.17
Dietary Pattern One	-0.38(-0.87 to -0.01)	-0.88(-1.07 to -0.34)	-0.55(-0.89 to -0.25)	-0.31(-0.78 to -0.01)	-0.08(-0.40 to 0.54)	<.0001

Dietary Pattern Two		-0.04(-0.50 to 0.56)	0.10(-0.36 to 0.75)	-0.31(-0.52 to 0.45)	0.03(-0.73 to 0.57)	-0.05(-0.42 to 0.58)	0.47
Couple-level characteristics							
Previous infertility examination		188(83.6)	45(81.8)	48(85.7)	51(87.9)	44(78.6)	0.56
Previous infertility treatment		107(51.7)	20(40.0)	25(47.2)	31(58.5)	31(60.8)	0.13
History of past pregnancy		86(37.4)	22(38.6)	20(35.1)	25(43.1)	19(32.8)	0.64
Primary infertility diagnosis							
Male factor		85(36.8)	21(36.8)	21(36.2)	20(34.5)	23(39.7)	0.93
Female factor	Diminished ovarian reserve	24(10.4)	8(14.0)	5(8.6)	5(8.6)	6(10.3)	
	Endometriosis	14(6.1)	4(7.0)	4(6.9)	4(6.9)	2(3.5)	
	Ovulatory	22(9.5)	5(8.7)	7(12.1)	8(13.8)	2(3.5)	
	Tubal disease	17(7.4)	5(8.8)	3(5.2)	5(8.6)	4(6.9)	
	Uterine	3(1.3)	1(1.8)	1(1.7)	1(1.7)	0(0)	
	Other disease	4(1.7)	2(3.5)	1(1.7)	0(0)	1(1.7)	
Unexplained		62(26.8)	11(19.3)	16(27.6)	15(25.9)	20(34.5)	
Initial stimulation protocol							
Protocol	Antagonist	35(15.2)	14(24.6)	3(5.2)	9(15.5)	9(15.5)	0.12
	Flare	22(9.5)	8(14.0)	5(8.6)	5(8.6)	4(6.9)	
	Luteal phase agonist	152(65.8)	33(57.9)	41(70.7)	39(67.2)	39(67.2)	
	Egg donor or cryo cycle	22(9.5)	2(3.5)	9(15.5)	5(8.6)	6(10.3)	
Initial mode of insemination; ICSI		122(59.2)	33(61.1)	29(60.4)	32(61.5)	28(53.9)	0.85

	Total	Dietary Pattern Two				
		Q1	Q2	Q3	Q4	P**

n	231	57	58	58	58	
Demographics characteristics						
Age(y)	36.8(33.4-40.0)	35.5(33.0-39.2)	37.7(34.1-39.9)	35.8(31.6-38.4)	38.0(34.0-41.9)	0.08
BMI(kg/m^2)	26.9(24.1-29.1)	27.4(25.6-30.0)	26.8(23.6-29.7)	26.0(23.4-28.5)	27.1(25.1-28.7)	0.04
Race, white	206(89.2)	54(94.7)	51(87.9)	50(86.2)	51(87.9)	0.43
Smoking, never smoker	153(66.2)	36(63.2)	34(58.6)	43(74.1)	40(69.0)	0.32
Education, college or higher	183(84.7)	37(71.2)	50(89.3)	49(92.5)	47(85.5)	0.02
Moderate-to-strenuous exercise(min/week)	165(60-390)	150(30- 360)	150(47-372)	239(84-420)	205(72-402)	0.29
Calories(kcal/day)	1934(1586-2384)	1664(1237-2077)	1794(1483-2131)	1891(1668-2204)	2568(2096-2900)	<0.001
Reproductive characteristics						
History of varicocele	19(8.2)	5(8.8)	3(5.2)	7(12.1)	4(6.9)	0.57
Female partner characteristics						
Age(y)	35.0(32.0-38.0)	35.0(32.0-38.0)	36.0(33.0-38.0)	33.5(32.0-38.0)	36.0(33.0-39.0)	0.36
BMI(kg/m^2)	23.1(21.0-25.7)	23.3(21.7-27.9)	22.8(21.1-24.8)	22.9(20.9-25.6)	23.1(20.7-25.7)	0.41
Race, white	194(84.4)	48(84.2)	50(86.2)	48(84.2)	48(82.8)	0.97
Smoking, never smoker	166(72.2)	39(68.4)	37(63.8)	46(80.7)	44(75.9)	0.18
Dietary Pattern One	-0.38(-0.87 to -0.01)	-0.36(-0.85 to 0.07)	-0.41(-0.92 to -0.05)	-0.40(-0.84 to -0.06)	-0.31(-0.86 to 0.10)	0.8
Dietary Pattern Two	-0.04(-0.50 to 0.56)	-0.44(-0.99 to -0.15)	-0.15(-0.56 to 0.42)	0.25(-0.31 to 0.47)	0.57(-0.05 to 1.16)	<0.001
Couple-level characteristics						
Previous infertility examination	188(83.6)	46(82.1)	44(77.2)	51(91.1)	47(83.9)	0.24
Previous infertility treatment	107(51.7)	25(48.1)	21(42.9)	30(56.6)	31(58.5)	0.39
History of past pregnancy	86(37.4)	24(42.1)	26(44.8)	15(26.3)	21(36.2)	0.2
Primary infertility diagnosis						
Male factor	85(36.8)	20(35.1)	21(36.2)	27(46.6)	17(29.3)	0.26

Female factor	Diminished ovarian reserve	24(10.4)	7(12.3)	5(8.6)	4(6.9)	8(13.8)	
	Endometriosis	14(6.1)	5(8.8)	3(5.2)	2(3.5)	4(6.9)	
	Ovulatory	22(9.5)	7(12.3)	4(6.9)	7(12.1)	4(6.9)	
	Tubal disease	17(7.4)	5(8.7)	7(12.1)	2(3.5)	3(5.2)	
	Uterine	3(1.3)	1(1.8)	1(1.7)	0(0)	1(1.7)	
	Other disease	4(1.7)	0(0)	0(0)	0(0)	4(6.9)	
Unexplained		62(26.8)	12(21.1)	17(29.3)	16(27.6)	17(29.3)	
Initial stimulation protocol							
Protocol	Antagonist	35(15.2)	9(15.8)	12(20.7)	5(18.6)	9(15.5)	0.77
	Flare	22(9.5)	4(7.0)	6(10.3)	7(12.1)	5(8.6)	
	Luteal phase agonist	152(65.8)	39(68.4)	33(56.9)	42(72.4)	38(65.5)	
	Egg donor or cryo cycle	22(9.5)	5(8.8)	7(12.1)	4(6.9)	6(10.3)	
Initial mode of insemination; ICSI		122(59.2)	28(53.9)	30(61.2)	31(57.4)	33(64.7)	0.71

*Data are presented as median (interquartile range) for continuous variables or n (%) for categorical variables.

** From the Kruskal-Wallis test for continuous variables and Fisher exact test for categorical variables except for primary infertility diagnosis and *in vitro* fertilization treatment protocol where the Chi-square test was used.

BMI: body mass index. Q:quartile

Table 2. Association between men's adherence to Dietary Pattern One and Two and semen parameters.

	Estimate (95% CI)	
	Pattern One	Pattern Two
Total	N=231 men	
Volume	-0.21(-0.48 to 0.04)	-0.16 (-0.38 to 0.05)
Total sperm count	1.54(-24.70 to 27.77)	-16.53(-38.41 to 5.35)
Sperm concentration	13.33(0.71 to 25.96) *	1.07(-9.42 to 11.57)
Total motility	0.17(-4.36 to 4.70)	-1.97(-5.73 to 1.80)
Progressive motility	0.64(-2.21 to 3.48)	-0.76(-3.12 to 1.60)
Morphology	0.03(-0.58 to 0.65)	0.11(-0.41 to 0.62)
Past examination	N=188 men	
Volume	-0.20(-0.49 to 0.08)	-0.11(-0.36 to 0.12)
Total sperm count	11.23(-17.55 to 40.01)	-11.87(-36.47 to 12.72)
Concentration	17.93(3.55 to 32.30) *	2.98(-9.26 to 15.22)
Total motility	1.12(-3.67 to 5.92)	-2.24(-6.31 to 1.83)
Progressive motility	1.15(-1.97 to 4.27)	-1.03(-3.68 to 1.62)
Morphology	0.40(-0.30 to 1.09)	0.16(-0.43 to 0.75)
Never examination	N=37 men	
Volume	-0.20(-0.84 to 0.43)	-0.36(-0.86 to 0.14)
Total sperm count	-52.28(-108.91 to 4.34)	-43.63(-88.17 to 0.92)
Concentration	-15.50(-37.52 to 6.52)	-13.02(-30.24 to 4.21)
Total motility	-6.03(-17.84 to 5.78)	-3.14(-12.37 to 6.09)
Progressive motility	-3.74(-9.82 to 2.34)	-1.82(-6.59 to 2.94)
Morphology	-2.11(-3.07 to -1.14) *	-0.14(-0.95 to 0.67)

* $P<0.05$

Supplementary material:

Supplemental Table 1. Baseline demographic and reproductive characteristics among included / excluded men.[*]

	Included	Excluded	P-value[**]
n	231	146	
Demographics, men			
Age (y)	36.8(33.4-40.0)	35.89(32.5-40.1)	0.77
BMI (kg/m2)	26.9(24.1, 29.1)	26.7(24.8-30.1)	0.32
Race, white	206(89.2)	124(85.5)	0.33
Smoking status, never	153(66.2)	107(73.9)	0.17
Education, college or higher	183(84.7)	55(83.3)	0.85
Reproductive history			
History of varicocele	19(8.2)	16(11.0)	0.23
Female partner characteristics			
Age (y)	35.0(32.0-38.0)	34.0(31.0-38.0)	0.06
BMI (kg/m2)	23.1(21.0-25.7)	23.2(20.6-27.0)	0.27
Race, white	194(84.4)	123(84.3)	1
Smoking Status	166(72.2)	112(76.7)	0.34
Couple-level characteristics			
History of past pregnancy	86(37.4)	71(48.6)	0.05
Previous infertility treatment	107(51.7)	74(57.8)	0.28

Primary infertility diagnosis				0.48
	Male factor		85(36.8)	45(30.8)
	Female factor	Diminished ovarian reserve	24(10.4)	21(14.4)
		Endometriosis	14(6.1)	7(4.8)
		Ovulatory	22(9.5)	20(13.7)
		Tubal disease	17(7.4)	7(4.8)
		Uterine	3(1.3)	2(1.4)
		Other disease	4(1.7)	0(0)
	Unexplained		62(26.8)	44(30.1)

*Data are presented as median (interquartile range) for continuous variables or n (%) for categorical variables.

** From unpaired t-test for continuous variables and Fisher exact test for categorical variables except for primary infertility diagnosis where the Chi-square test was used.

BMI: body mass index. Q: quartile.

Supplemental Table 2. Factor loadings for food groups* in dietary patterns identified by principal component analysis among 231 couples undergoing infertility treatment.

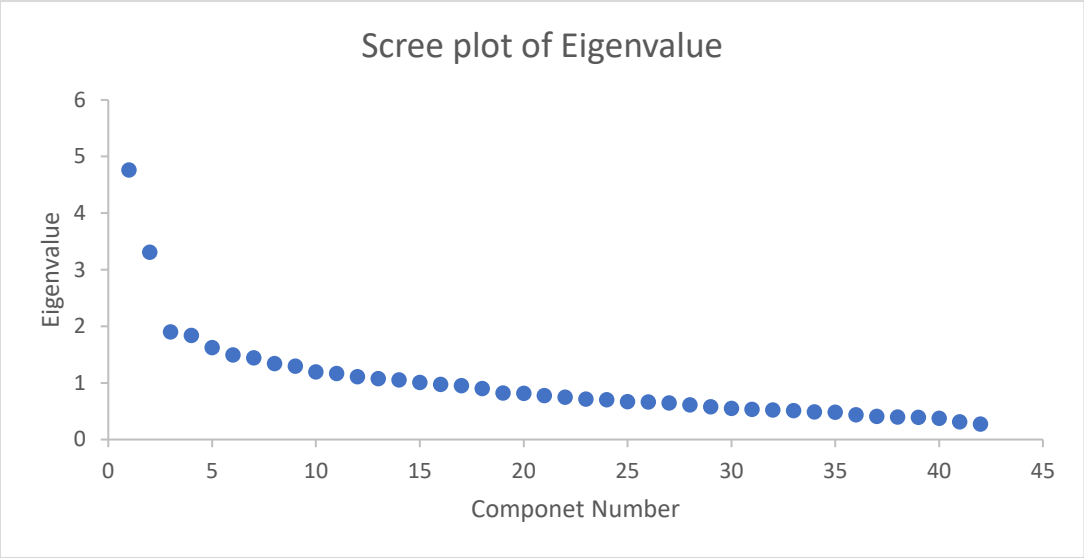
Food group	Pattern One	Pattern Two
Processed meat	0.56	-
Red meat	0.59	-
Butter	0.41	-
High fat dairy	0.45	-
Beer	0.46	-
Fruit	-	0.63
Cruciferous vegetables	-	0.59
Yellow vegetables	-	0.69
Tomatoes	0.32	0.52
Leafy green vegetables	-	0.65
Legumes	-	0.63
Soy food and soymilk	-	0.33
Other vegetables	-	0.75
Potatoes	-	0.36
French fries	0.63	-
Whole grains	-	0.36
Refined grains	0.44	-
Pizza	0.39	-
Snacks	0.45	-
Nuts and Nut butters	-	0.33
High-energy drinks	0.39	-
Low-energy drinks	0.37	-
Mayonnaise	0.38	-
Chowder or cream soup	0.54	-
Sweets and desserts	0.37	-
Condiments	0.36	-
Added salts	0.37	-
Variance explained	11.40%	7.90%

*Food groups with loadings <|0.3| are listed in the methods section but not shown here.

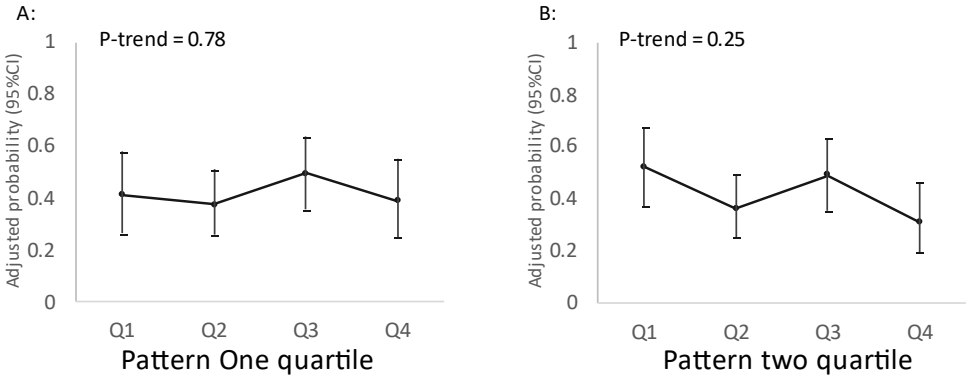
Supplemental Table 3. Distribution of semen quality parameters among study participants.

Semen parameter	Median [25th - 75th percentile]	Proportion of men below WHO reference value, %	WHO Reference value (30)
Volume (ml)	2.7[1.9-3.4]	13.3	1.5ml
Total sperm count ($\times 10^6$)	137.7[69.1-227.4]	7.8	39×10^6
Sperm concentration ($\times 10^6$ /ml)	54.7[26.5-93.6]	9.7	15×10^6 / mL
Total motility (%)	45.9[27.2-61.9]	39.8	40% motile
Progressive motility (%)	25.5[15.0-36.0]	62.5	32% motile
Normal Morphology (%)	6.0[4.0-8.4]	23.8	4% normal

Supplemental Figure 1. Scree plot of principal component analysis.

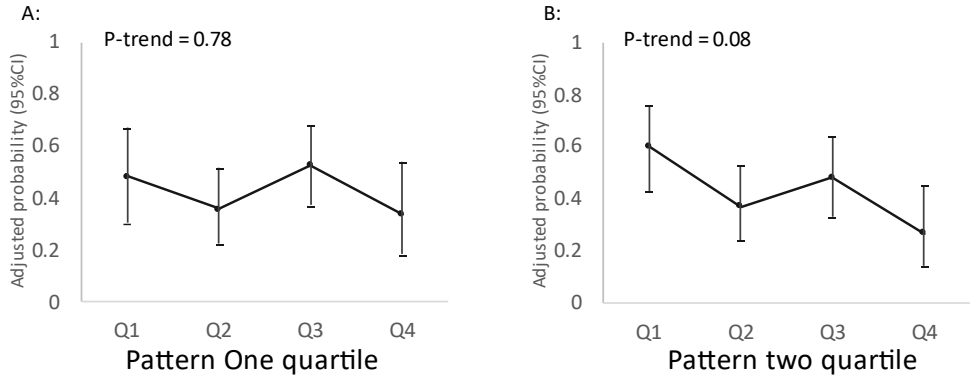


Supplemental Figure 2. Men's adherence to dietary pattern one and two in relation to live birth with IVF treatment with the initial multivariable-adjusted model among couples whose female partner complete FFQ (N = 213 couples, 367 cycles) [*].



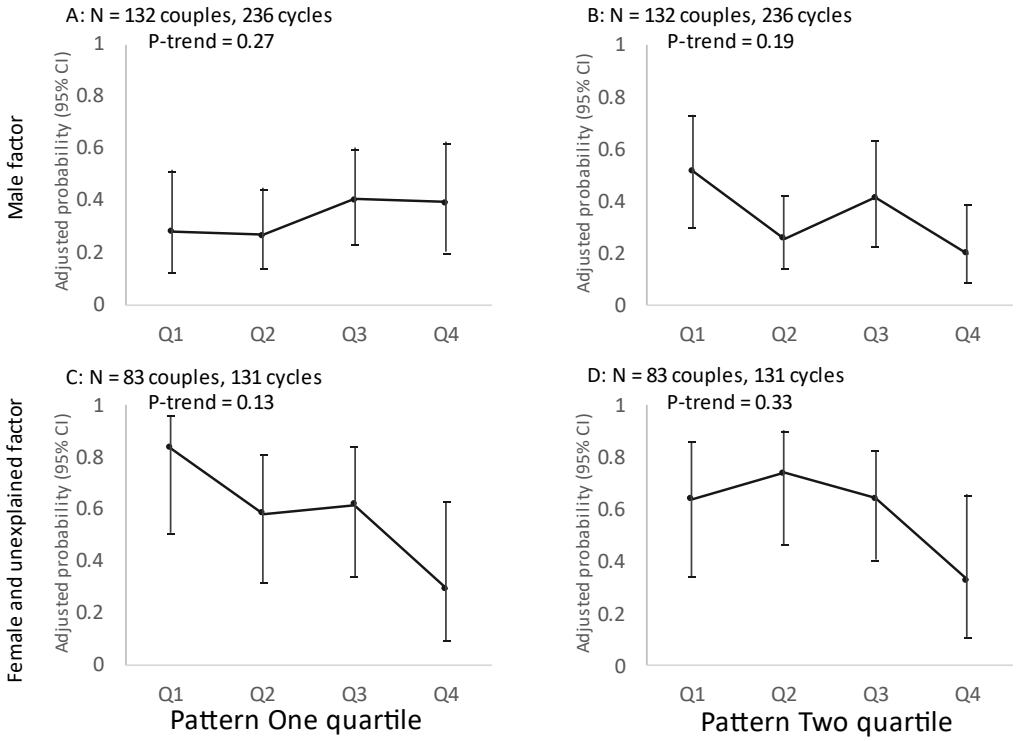
[*] Adjusted for men's age, total calorie intake, BMI, race, smoking status, education level, and moderate-to-vigorous physical activity; women's age and BMI; primary infertility and treatment protocol. Q: quartile.

Supplementary Figure 3. Men's adherence to dietary pattern one and two in relation to live birth with IVF restricted to the first treatment cycle for each couple (N = 213 couples, 213 cycles).*



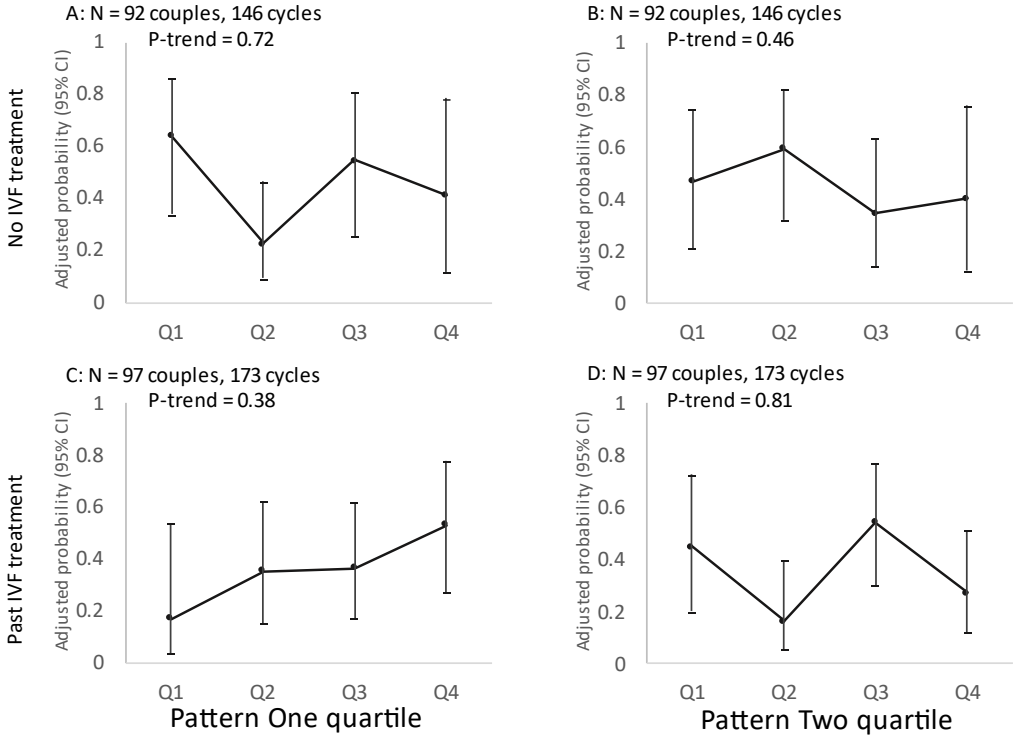
* Adjusted for men's age, total calorie intake, BMI, race, smoking status, education level and moderate-to-vigorous physical activity; women's age, BMI, and adherence to dietary pattern one and two; primary infertility diagnosis and treatment protocol. Q: quartile.

Supplemental Figure 4. Men's adherence to dietary pattern one and two in relation to live birth with IVF treatment stratified by primary infertility diagnosis (male factor or female and unexplained factor).*



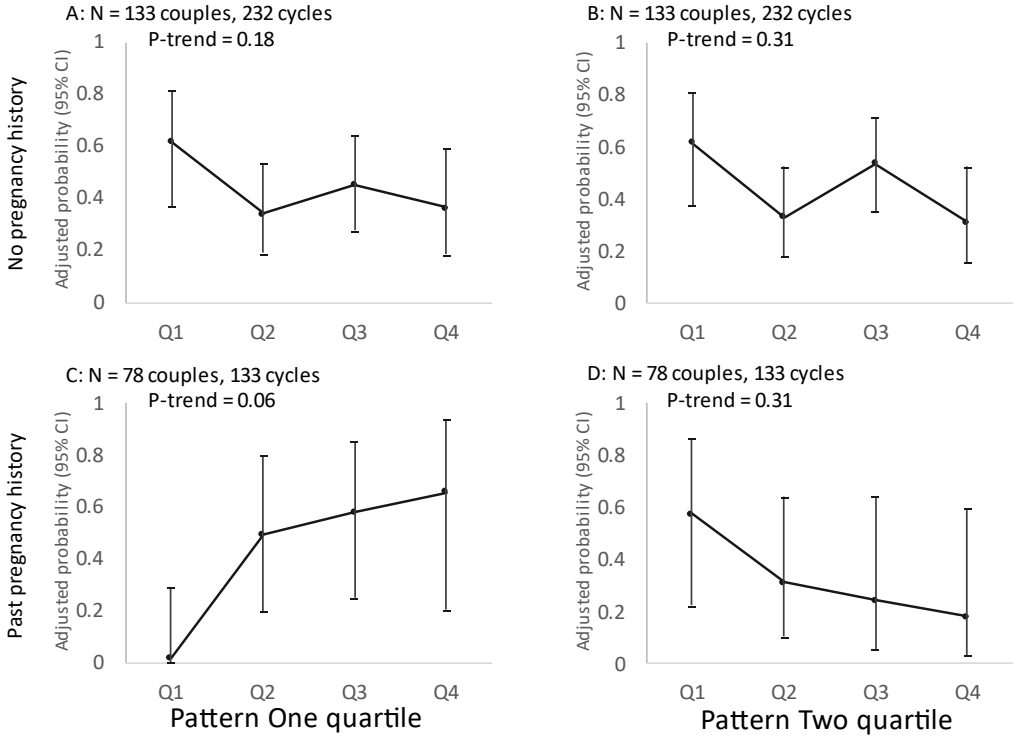
* Adjusted for men's age, total calorie intake, BMI, race, smoking status, education level, and moderate-to-vigorous physical activity; women's age, BMI, and adherence to dietary pattern one and two; primary infertility diagnosis and treatment protocol. Q: quartile.

Supplemental Figure 5. Men's adherence to dietary pattern one and two in relation to live birth with IVF treatment stratified by IVF treatment history.*



* Adjusted for men's age, total calorie intake, BMI, race, smoking status, education level, and moderate-to-vigorous physical activity; women's age, BMI, and adherence to dietary pattern one and two; primary infertility diagnosis and treatment protocol. Q: quartile.

Supplemental Figure 6. Men's adherence to dietary pattern one and two in relation to live birth with IVF treatment stratified by pregnancy history.*



* Adjusted for men's age, total calorie intake, BMI, race, smoking status, education level, and moderate-to-vigorous physical activity; women's age, BMI, and adherence to dietary pattern one and two; primary infertility diagnosis and treatment protocol. Q: quartile.

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PAPER 2 (Submitted to Fertility and Sterility, status: under review)

A dietary score representing the overall relation of men's diet with semen quality in relation to outcomes of infertility treatment with assisted reproduction

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Abstract:

Objective: To examine the impact of men's diet on outcomes of infertility treatment with assisted reproductive technology (ART) using an empirical score representing the relation of diet with semen quality.

Design: Prospective cohort study.

Setting: Fertility center at an academic medical center.

Patients: We included 296 men (688 semen samples) to identify an empirical dietary pattern and 231 couples (406 ART cycles) to investigate the association of this diet pattern with ART outcomes.

Intervention: Men's diet was assessed at baseline using a validated questionnaire. An empirical dietary pattern reflecting the overall relation of diet with semen quality was identified using reduced rank regression.

Main outcome measures: The primary outcome was live birth per treatment cycle. Secondary outcomes were fertilization, implantation, and clinical pregnancy.

Results: Men had a median baseline age and BMI of 36.8 years and 26.9 kg/m^2, respectively. Although the empirical diet pattern was significantly associated with all semen parameters, the empirical diet score was not related to any clinical outcome of infertility treatment following ART. The adjusted probabilities of relevant clinical outcomes in the lowest and highest quartile of the empirical score were 0.62 (0.50-0.73) and 0.55 (0.45-0.66) for implantation; 0.57 (0.46-0.69) and 0.50 (0.40-0.61) for clinical pregnancy; and 0.49 (0.37-0.62) and 0.36 (0.25-0.48) for live birth. Analyses excluding couples with a diagnosis of male factor infertility and, separately, excluding ICSI cycles yielded similar results.

Conclusions: A dietary score representing the overall association of diet with semen quality parameters was not associated with ART outcomes.

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Trial registration number: Not applicable

Keywords: Male diet, semen quality, reduced rank regression, ART, live birth

Introduction:

Infertility is an increasingly important medical condition worldwide, affecting over 15% of couples of reproductive age (1). While much of the focus on understanding the causes of infertility focuses on women, male factors account for approximately half of the infertility burden (2). Standard semen analysis is not only an important biomarker of spermatogenesis and testicular function but is also the cornerstone for the clinical diagnosis of male factor infertility (2,3). However, it is known that semen analysis is not a perfect predictor of couples' fertility, both in couples attempting conception on their own and in couples attempting conception with medical assistance (4,5).

Men's diet has been increasingly recognized as a potentially modifiable factor influencing semen quality. For example, intakes of ω-3 fatty acids (6,7), Coenzyme Q10 (8), and carnitine (9), as well as foods such as fish, seafood, poultry, vegetables, fruits (10–13), nuts and whole cereals (14–16), have been positively related to semen quality. Similarly, adherence to healthier dietary patterns like the Mediterranean or prudent diet has been positively associated with semen parameters, whereas the opposite appears to be the case for adherence to unhealthy dietary patterns such as western pattern (10–13). However, there is little data evaluating the impact of men's diet on a couple's fertility. This is a particularly important knowledge gap as some data suggests that associations between diet and semen quality do not necessarily translate into associations with couple-based outcomes, such as fertility (17–21).

The goal of this study was to evaluate the extent to which men's dietary factors associated with semen quality are also predictive of couples' infertility treatment outcomes. To achieve this goal, we empirically derived a dietary score capturing the overall association of

diet with semen quality and then examined this score in relation to the probability of achieving a live birth in the course of infertility treatment with assisted reproductive technology (ART).

Materials and Methods:

Study population

Couples presenting to the Massachusetts General Hospital (MGH) Fertility Center were invited to enroll in the Environment and Reproductive Health (EARTH) Study, a prospective cohort study aimed at evaluating the impact of environmental and nutritional factors (22) on fertility and pregnancy outcomes. Women aged 18–45 years and men 18–55 years without a history of vasectomy and who had not been administered any hormonal treatment at the enrollment of study were eligible. A total of 982 women, 553 men, and 513 couples enrolled between April 2004 and December 2019. Participants were encouraged, but not required, to take part in the study as a couple. All participants who joined signed written informed consent. Study staff administered a baseline questionnaire, including demographics, medical, reproductive, occupation histories, and lifestyle, and conducted anthropometric measurements. Participants also provided blood and urine specimens during the first study visit (22). They also completed a Food Frequency Questionnaire (FFQ), introduced in April 2007, to assess habitual diet of participants. For the present study, we used data of two partially overlapping subgroups of patients in the EARTH study. For analyses aimed at identifying empirical diet patterns related to semen, we included all men who enrolled in the study from April 2007 to June 2019, completed the FFQ and produced at least one semen sample. We excluded men with azoospermia (n=7 men) and

men with missing values in any semen parameter or abstinence time (n=46 men), leaving 296 men (688 semen samples) available for analysis. For analyses aimed at evaluating the role of men's diet on ART outcomes, we used data from all couples where the male partner completed the FFQ and the female partner underwent at least one ART cycle from April 2007 to April 2018. We excluded couples with missing key covariate data (n=1 couple), leaving 231 couples (406 ART cycles) available for analysis. The Institutional Review Boards of MGH and the Harvard T.H. Chan School of Public Health approved the study.

Semen analysis

Men provided semen specimens on-site via masturbation. A 48-hour abstinence period before sample production was recommended and actual abstinence time was recorded for each sample. Semen samples were maintained at 37 °C and allowed to liquefy. All assessments were performed within 30 min of collection following the 2010 WHO manual guidelines (3). Ejaculate volume was estimated by sample weight, assuming a semen density of 1g/ml. Sperm concentration and motility were assessed by computer-assisted semen analysis (CASA; 10HTM-IVOS, Hamilton-Thorne Research, Beverly, MA) (23). Motile spermatozoa were defined according to the WHO four-category scheme: rapid progressive, slow progressive, non-progressive and immotile. Total sperm count (million/ejaculate) was calculated by multiplying ejaculated volume by sperm concentration. Sperm morphology (% normal) was assessed on two slides per specimen (with a minimum of 200 cells assessed per slide) via a microscope with an oil-immersion ×100 objective (Nikon, Tokyo, Japan). Strict Kruger scoring criteria were used to classify men as having normal or below normal morphology (24).

Dietary assessment and dietary score

Diet was assessed using a previously validated FFQ of 131 foods and beverages (25,26). Participants were asked to report how often, on average, during the previous year, they consumed each food item. Response options ranged from never or less than once per month to six or more times per day. The individual foods and beverage items were categorized into 42 pre-defined foods and beverages groups based on those proposed by Hu and colleagues (27).

Clinical procedures

Women underwent one of three ovarian stimulation protocols for fresh *in vitro fertilization* (IVF) protocol: (1) GnRH-antagonist protocol; (2) follicular phase GnRH-agonist/flare-up protocol; or (3) luteal phase GnRH-agonist protocol. Embryologists classified oocytes as germinal vesicle, metaphase I, metaphase II (MII), or degenerated. MII oocytes underwent conventional insemination (IVF) or intracytoplasmic sperm injection (ICSI) as clinically indicated. Embryologists evaluated fertilization status on day 1 after fertilization based on the presence of two pronuclei (2PN). Fertilization rate was defined as the number of 2PN embryos divided by the number of MII oocytes. Embryo transfer was performed either following stimulation and retrieval or following thawing of cryopreserved embryos (19,22). Clinical outcomes were evaluated among women who underwent embryo transfer. An elevation of serum β-hCG level greater than 6 m IU/mL at approximately two weeks after embryo transfer was defined as successful implantation. The presence of an intrauterine gestational sac observed on ultrasonographic evaluation at around 6

gestational weeks was considered as a clinical pregnancy. Live birth was defined as the birth of a neonate at or after 24 weeks of gestation.

Statistical Analysis

To evaluate the overall impact of diet on all semen quality parameters simultaneously, we conducted a reduced rank regression (RRR) analysis (28). RRR is a statistical procedure that is aimed at dimension reduction by simultaneously modeling the association of a set of predictors with a group of related outcome measures with the goal of obtaining a single or a limited number of summary response measures (factors). In nutritional epidemiology, RRR has been used to identify how diet mediates health effects through specific biological pathways by modeling the simultaneous association of multiple dietary factors on multiple biomarkers of the same underlying biologic process (e.g., inflammation) (29,30). In this case, we used semen quality parameters as biological intermediates between diet and fertility. Before applying, to decrease variability in semen quality due to differences in abstinence time, we adjusted each semen parameter by abstinence time using the residual method in a mixed linear regression model that included linear, quadratic, and cubic terms for abstinence time. The residuals for each man were then averaged to obtain a single time-integrated and abstinence time-independent measure of semen quality for each man. Then, we conducted a RRR analysis where the 42 pre-defined foods and beverages groups were the predictive variables, and the mean of the residual of each semen parameter (ejaculate volume, total sperm count, semen concentration, total motility, progressive motility, and percentage of sperm normal morphology) were response variables. The first factor from

this model was retained and interpreted as an empirical score capturing the overall relation of diet with semen quality.

Men were categorized into quartiles according to their empirical dietary score. Differences in the proportion or median of demographic, reproductive, and nutritional characteristics across quartiles of the empirical diet score were evaluated with the Kruskal-Wallis test for continuous variables and the Chi-square or Fisher exact test for categorical variables.

To corroborate that the solution from RRR model captured the overall association of diet with semen quality, we fit six separate linear regression models where the exposure of interest was the empirical dietary score, and the outcome of interest was each semen parameter (ejaculate volume, total sperm count, total motility, progressive motility and the percentage of sperm normal morphology). Total sperm count and sperm concentration were log-transformed to improve more closely approach a normal distribution. To allow direct comparisons of the magnitude of relation of the diet score across all outcomes, we standardized the diet score and each of the outcomes by dividing each variable by its standard deviation. Results from these models can therefore be interpreted as the difference in each semen parameter, in standard deviation units, associated with a 1 standard deviation increase in the empirical diet score.

Then, to evaluate the association between the male diet score with ART outcomes, we fit multivariable generalized linear mixed models with random intercepts to account for repeated ART cycles per couple while adjusting for potential confounders. A binomial distribution and logit link function were specified for fertilization rate and clinical outcomes (implantation, clinical pregnancy, and live birth). The primary outcome of this study was the probability of live birth per initiated treatment cycle. Secondary outcomes

were fertilization rate, and the probabilities of implantation, and clinical pregnancy during the course of infertility treatment with ART. We used population marginal means to present results as probabilities and their corresponding 95% confidence intervals (95% CI) adjusted for all covariates in the model (31). We evaluated the linear trend across the quartiles of the dietary score by modeling the dietary score as a continuous variable. We chose the confounders using previous scientific knowledge and by assessment of the difference in patients' baseline characteristics across the quartiles. The primary multivariable-adjusted model included terms for men's and women's age, men's total calorie intake per day, total physical activity (min/week), couples' primary infertility diagnosis, and treatment protocol and, in models for fertilization rate as the outcome, type of insemination (ICSI vs. conventional IVF). The second model included additional terms for men's and women's body mass index (BMI). For the second model, we had missing data on BMI for two men and three women. We decided to use complete data in the analysis resulting in the exclusion of 5 cycles. Using cross-product terms, we evaluated effect modification by insemination mode (ICSI vs. IVF).

Last, to evaluate the robustness of our findings, we performed a series of sensitivity analyses. We first performed analyses restricted to couples without a diagnosis of male factor infertility and, separately, excluding ICSI cycles. Then we repeated the RRR analysis without adjusting semen parameters for abstinence time and using only semen samples produced within the WHO-recommended abstinence period of 2-7 days (3) and evaluated the relation of this new empirical diet score with all clinical outcomes. All analyses were performed using SAS university edition with VirtualBox version 6.1.10.

Results:

In total, we included 296 men (688 semen samples) in analyses aimed at identifying the empirical dietary pattern and 231 couples (406 ART cycles) in analyses aimed at evaluating the association of this diet pattern with ART outcomes. Supplemental Table 1 shows the distribution of semen quality parameters among study participants. Not surprisingly, men in the study had a high proportion of samples with values below 2010 WHO reference values, particularly for sperm motility (39.8% for total motility and 62.5% for progressive motility) (Supplemental Table 1). Figure 1 shows the results of the RRR model. Food groupings with positive factor loadings are positively associated with semen quality, whereas negative factor loadings have the opposite interpretation. As expected, the empirical diet pattern was significantly associated with all semen quality parameters. A one SD increase in empirical dietary score was associated with lower ejaculate volume (-0.10 standard units [95% CI: -0.17 to -0.04]) and to higher total sperm count (0.12 standard units [0.06 to 0.19]), sperm concentration (0.17 standard units [0.10 to 0.24]), total sperm motility (0.14 standard units [0.07 to 0.20]), progressive sperm motility (0.08 standard units [0.01 to 0.15]), and normal sperm morphology (0.18 standard units [0.11 to 0.25]) (Figure 2).

We then evaluated the relation of the empirical score with ART outcomes. The median (IQR) age and BMI of women were 35.0 years (32.0-38.0) and 23.1 kg/m^2 (21.0-25.7). The corresponding values for men were 36.8 (33.4-40.0) years and 26.9 (24.1-29.1) kg/m^2. The empirical diet score was positively associated with total energy intake and physical activity (Table 1). In addition, the frequency of male factor infertility as the primary infertility diagnosis decreased with increasing levels of the empirical score. The

distribution of the initial stimulation protocol also differed according to quartiles of the empirical score (Table 1). No other demographic, nutritional, or reproductive characteristics were associated with the score (Table 1).

We found no association between the empirical dietary score with fertilization rate overall nor when examined separately in IVF and ICSI cycles (Figure 3). Similarly, the empirical score was unrelated to the probability of implantation, clinical pregnancy, and live birth per initiated treatment cycle (Figure 4). The adjusted probabilities of implantation, clinical pregnancy, and live birth for couples with the primary model in the lowest and highest quartile of the empirical score were 0.62 (0.50-0.73) and 0.55 (0.45-0.66), 0.57 (0.46-0.69), and 0.50 (0.40-0.61), and 0.49 (0.37-0.62) and 0.36 (0.25-0.48), respectively. Results were nearly identical after additional adjustment for men's and women's BMI (data not shown). Also, we found no evidence of effect modification by type of insemination.

We found no evidence of an association between the empirical diet score with any clinical ART outcome in analyses excluding couples with a primary diagnosis of male factor infertility (Supplemental Figures 1 and 2) and in analyses excluding ICSI cycles (Supplemental Figure 3). Similarly, when we revised the RRR model to not account for abstinence time (Supplemental Figure 4), the revised empirical diet score was also unrelated to all ART outcomes evaluated (Supplemental Figures 5 and 6).

Discussion:

We investigated the association between men's diet, using an empirical dietary score capturing the overall impact of diet on semen quality, and ART outcomes among subfertile

couples undergoing infertility treatment at an academic fertility center. We found that, despite being associated with all standard semen parameters, this empirical dietary score was not related to any ART outcome. Our findings suggest that the extent to which diet impacts semen quality among men in sub-fertile couples does not influence ART outcomes, including fertilization, implantation, clinical pregnancy, and live birth. More broadly, these data suggest that in the setting of infertility treatment with ART, any impact that men's diet – and possibly other environmental factors – may have on couple-based outcomes are unlikely to be a result of their effect on semen quality parameters.

Numerous studies have reported that men's diet has an impact on semen quality or other biomarkers of testicular function. In general, healthier dietary patterns, such as the Mediterranean diet patten which is characterized by higher intake of olive oil, fruits, vegetables, fruit, seafood, poultry and whole grains, have been associated with favorable semen quality in observational studies among healthy and subfertile men (32–34), and more recently in a randomized controlled trial (RCT) among healthy men (35). Another dietary factor with strong evidence of benefit is intake of long-chain n-3 fatty acids. For example, in a large study of military recruits in Denmark, use of fish oil supplements was associated with higher semen volume, total sperm count, and testis size, and lower follicle-stimulating hormone and luteinizing hormone levels (36). These findings are similar to those of RCTs of fish oil supplementation among subfertile men (37) and to RCTs of supplementation with nuts, which are also rich in n-3 fatty acids, among young healthy men (14,15).

Nevertheless, the literature linking men's diet to couple-based outcomes, such as fertility, is scant and inconsistent. On one hand, studies in independent populations have

documented inverse associations of men's intake of sugary beverages with both semen quality (38) and fertility (39). Similarly, studies in independent populations have documented positive associations of men's fish intake with semen quality (40) and fertility (18). Of note, in these cases, the associations of men's diet with a couple's fertility were documented in studies of pregnancy planners without a history of infertility. The picture in studies among couples undergoing infertility treatment is not as clear. For example, we have previously reported that men's intake of processed meats, dairy, soy, and carotenoids were associated to semen quality (21,40–42) but not to outcomes of infertility treatment with ART (17,19,20,43). Conversely, we have found associations between men's intakes of alcohol, caffeine, and vitamin C with ART outcomes in the absence of an association with semen quality in the same population (43,44). Clearly, it is important that additional studies evaluate the extent to which predictors of semen quality overlap with predictors of couple-based outcomes like fertility, both in couples attempting conception naturally and with medical assistance.

It is important to consider the study findings in light of their strengths and limitations. First and most saliently, the study was conducted among subfertile couples undergoing infertility treatment. As a result, the frequency of men with semen quality below WHO reference limits was higher than expected in a population of pregnancy planners without a history of infertility. However, sensitivity analyses excluding couples with a primary infertility diagnosis of male factor showed nearly identical results suggesting that the overrepresentation of men with poor semen quality alone does not explain the findings. Possibly of greater relevance is the possibility that infertility treatment with ART itself may completely negate any effects that environmental factors,

including diet, could have on a couple's fertility by influencing semen quality. This interpretation is consistent with the scant evidence to date on the relation between diet and a couple's fertility described above. In other words, given the stringent sperm selection procedures built into ART, especially ICSI, any effect that diet or other environmental factors may have on a couple's chances of conceiving is unlikely to reflect environmental impacts on semen quality. In fact, the results of the sensitivity analysis excluding ICSI cycles suggest that even conventional IVF may pose enough selective pressure on sperm to the point of nullifying the effect that environmental and behavioral factors may have on fertility through their effect on semen quality. Therefore, it is unclear whether findings from this study can be generalized to couples trying to conceive without ART. Moreover, our findings further highlight that bulk semen parameters are far from perfect biomarkers of men's reproductive potential (4,5), and it is thus important to examine whether other characteristics of sperm such as DNA integrity (45–47), RNA elements (48), proteomics (49,50), or others yet to be identified, are better able to capture how men's environment and behavior influence a couple's fertility in the general population and in the setting of infertility treatment. Second, this study had a limited number of couples, therefore the statistical power to detect any associations between diet and a couple's fertility could have been constrained. Third, we need to consider misclassification and measurement error in diet, which is a concern even when using extensively validated questionnaires. Nevertheless, this problem would apply uniformly to all outcomes. Hence, since the empirical diet score was predictive of semen quality in the same group of men, it is unlikely that measurement error alone is responsible for the lack of association with clinical ART outcomes.

There are also important strengths of this study. The use of a completely agnostic and data-driven approach to characterize the impact on men's diet on semen quality as our exposure variable has several advantages. First, it eliminates the impact of any prior beliefs on how diet might impact have on a couple's fertility. Second, given that decision-making on male factor infertility, including lifestyle recommendations patients may receive, is driven by the current knowledge on predictors of semen quality parameters, this approach approximates the type of advice men in couples facing difficulties conceiving may receive from their physicians. The availability of preconception information on the male partner, which, unfortunately, remains a rarity in studies of fertility, is an important strength of the study. Additional strengths include the prospective study design with a live birth rate as a primary outcome and complete follow-up of study participants for all study outcomes.

In conclusion, we found that an empirical dietary score capturing the overall impact of diet on semen quality was not related to infertility treatment outcomes with ART. Given that ART incorporates robust sperm selection procedures, it is possible that ART itself may negate the effects of environmental factors on a couple's ability to conceive with these treatments that are mediated through semen quality. As a result, it is unclear to what extent these findings can be generalized to couples attempting conception without medical assistance. In addition, these results further highlight the limitations of semen quality parameters as a predictor of a couple's fertility. Additional studies are necessary to understand how men's diet, environment, and behaviors impact on a couple's fertility, naturally and with medical assistance, and the extent to which men's reproductive potential can be captured through biomarkers other than bulk semen parameters.

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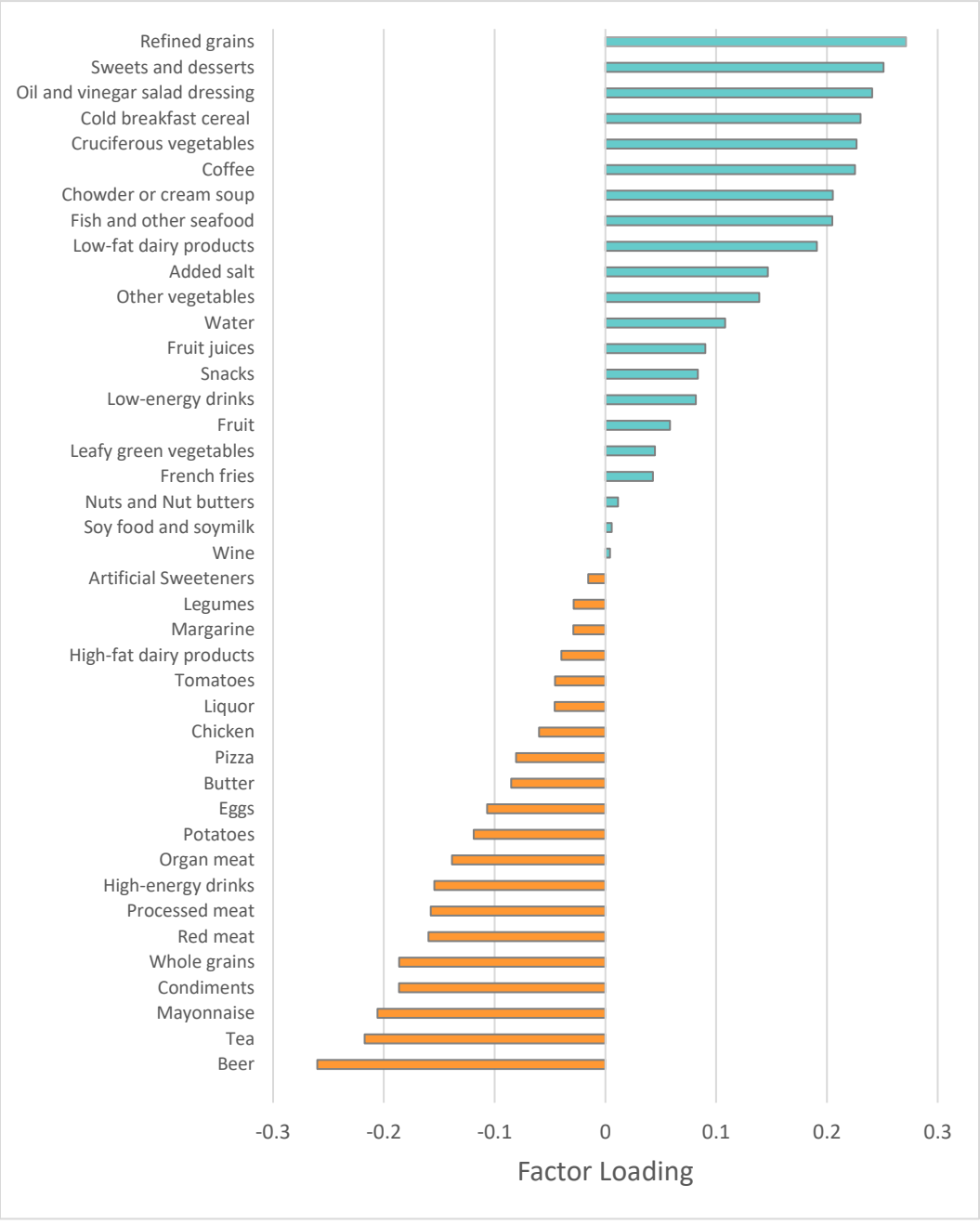
Conflict of interest:

No conflict of interest for Makiko Mitsunami, Albert Salas-Huetos, Lidia Mínguez-Alarcón, Jill A. Attaman, Jennifer B. Ford, Martin Kathrins, Irene Souter and Jorge E. Chavarro.

Figures:

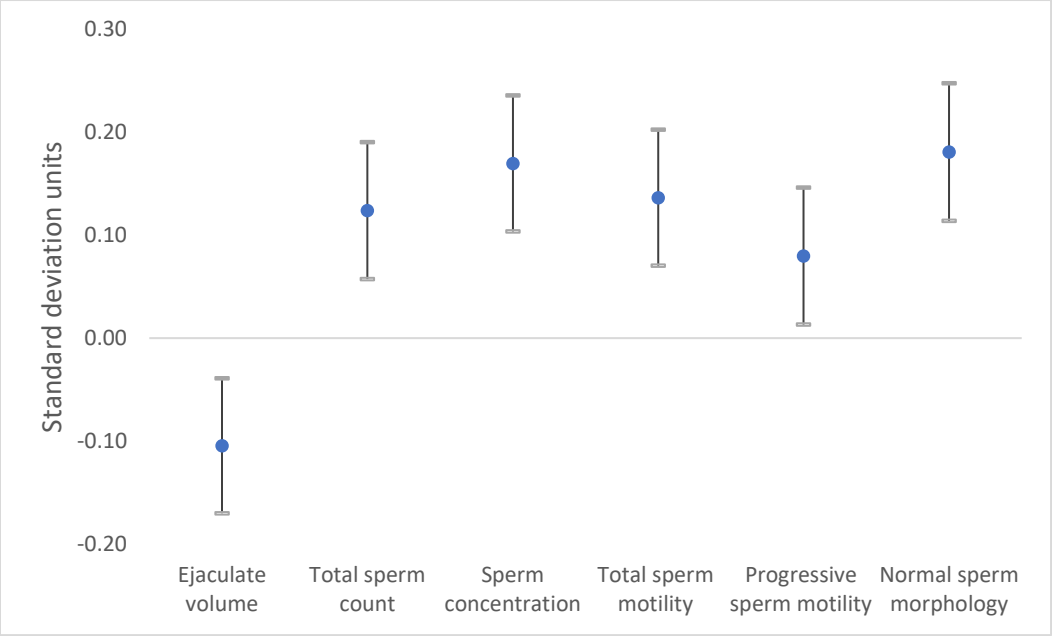
Figure 1. Factor loadings for reduced rank regression with food groups as predictive variables and semen parameters as response variables.*



* Turquoise bar: Positive factor loading food groups.

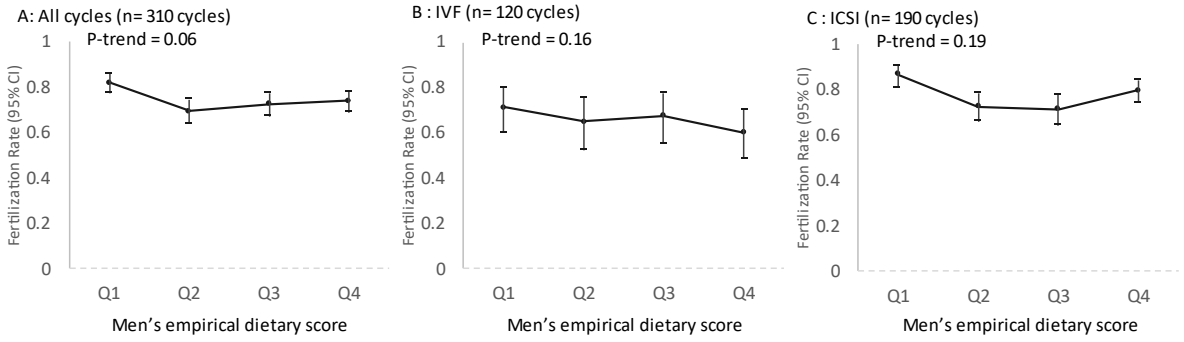
Orange bar: Negative factor loading food groups.

Figure 2. Association between empirical dietary pattern and individual semen quality parameters.*



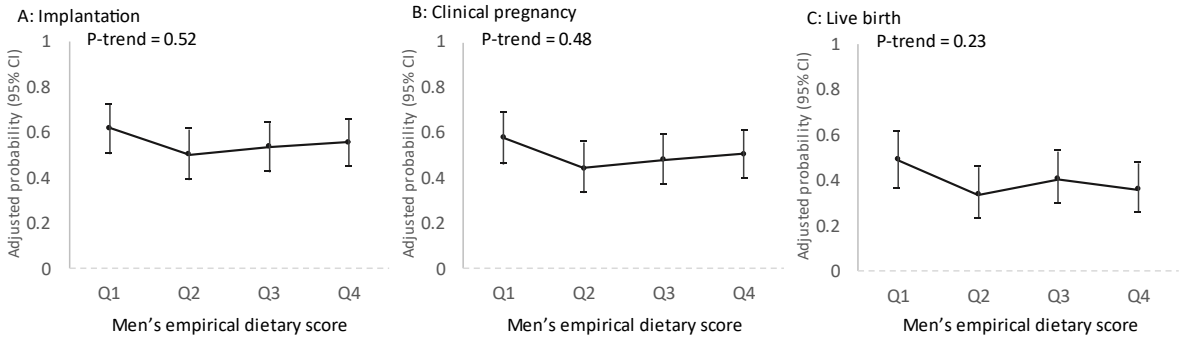
*All semen parameters and the empirical diet score have been standardized to allow direct comparison of the magnitude of association between the score across semen parameters.

Figure 3. Men's empirical dietary score in relation to fertilization rate, overall (A) and in IVF (B) or ICSI (C) cycles. *



* Adjusted for men's age, women's age, men's total calories intake, total exercise, ICSI (yes/no), primary infertility diagnosis and stimulation protocol. Stratified models (B and C) do not include a term for ICSI. Q: quartile.

Figure 4. Men's empirical dietary score in relation to clinical outcomes of infertility treatment with ART (N = 231 couples, 406 cycles). *



* Adjusted for men's age, women's age, men's total calories intake, total exercise, primary infertility diagnosis and stimulation protocol. Q: quartile.

Table:

Table 1. Baseline demographic, nutritional and reproductive characteristics of study participants, overall and by quartiles of the empirical dietary score. *

		Total	Q1	Q2	Q3	Q4	P-value**
Empirical dietary score			-0.33 to 0.43	0.44 to 0.78	0.79 to 1.07	1.08 to 2.98	
n		231	57	58	58	58	
Demographics, men	Age(y)	36.8(33.4-40.0)	37.2(34.4-40.0)	37.4(33.5-40.5)	35.9(31.9-39.2)	37.2(34.1-40.4)	0.45
	BMI (kg/m^2)	26.9(24.1-29.1)	27.4(24.8-30.0)	26.7(23.8-28.7)	26.7(24.5-29.3)	26.7(23.7-28.6)	0.36
	Race(white)	206(89.2)	49(86.0)	51(87.9)	53(91.4)	53(91.4)	0.75
	Smoking Status (Never smoker)	153(66.2)	41(71.9)	38(65.5)	35(60.3)	39(67.2)	0.63
	Education (College or higher)	183(84.7)	43(84.3)	44(84.6)	45(79.0)	51(91.1)	0.36
	Total physical activity(min/week)	347(150-629)	270(90-612)	210(84-510)	472(252-750)	372(221-600)	0.005
	Calories(kcal/day)	1934(1586-2384)	1906(1547-2189)	1794(1341-2221)	1910(1571-2384)	2233(1886-2724)	0.0003
Reproductive history	History of varicocele	19(8.2)	5(8.8)	6(10.3)	5(8.6)	3(5.2)	0.81
	Previous infertility examination	188(83.6)	46(82.1)	49(86.0)	49(86.0)	44(80.0)	0.79
	Previous infertility treatment	107(51.7)	21(40.4)	29(54.7)	29(55.8)	28(56.0)	0.31
	History of past pregnancy	86(37.4)	19(33.3)	23(39.7)	17(29.8)	27(46.6)	0.23
Primary infertility diagnosis	Male factor	85(36.8)	28(49.1)	28(48.3)	15(25.9)	14(24.1)	0.10
	Female factor	84(36.4)	20(35.1)	19(32.8)	22(37.9)	23(40.0)	

	Unexplained	62(26.8)	9(15.8)	11(19.0)	21(36.2)	21(36.2)	
Initial stimulation protocol	Antagonist	35(15.2)	6(10.5)	13(22.4)	8(13.8)	8(13.8)	0.05
	Flare	22(9.5)	11(19.3)	3(5.2)	4(6.9)	4(6.9)	
	Luteal phase agonist	152(65.8)	38(66.7)	38(65.5)	40(69.0)	36(62.1)	
	Cryo/donor	22(9.5)	2(3.5)	4(6.9)	6(10.3)	10(17.2)	
Demographics, female partner	Age(y)	35.0(32.0-38.0)	35.0(33.0-38.0)	36.0(33.0-38.0)	34.5(32.0-37.0)	35.5(32.0-39.0)	0.45
	BMI (kg/m2)	23.1(21.0-25.7)	23.5(21.6-26.2)	23.2(21.6-25.5)	22.2(20.1-24.2)	22.8(21.1-25.4)	0.15
	Race(white)	194(84.4)	47(82.5)	47(81.0)	53(93.0)	47(81.0)	0.19
	Smoking Status (Never smoker)	166(72.2)	39(68.4)	38(65.5)	44(77.2)	45(77.6)	0.37
	Dietary score for women	0.82(0.57-1.10)	0.76(0.54-1.01)	0.80(0.56-1.14)	0.79(0.51-1.14)	0.90(0.66-1.13)	0.46

*Data are presented as median (interquartile range) for continuous variables or n (%) for categorical variables.

** From the Kruskal-Wallis test for continuous variables and Fisher exact test for categorical variables except for primary infertility diagnosis and in vitro fertilization treatment protocol where the Chi-square test was used.

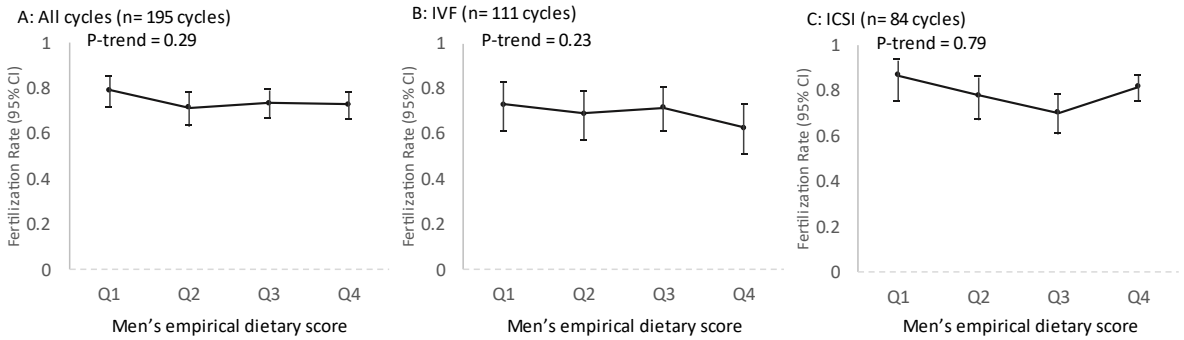
Q: quartile, BMI: body mass index, kcal: kilocalorie, and cryo: cryopreservation embryo

Supplementary material:

Supplemental Table 1. Distribution of semen quality parameters among study participants.

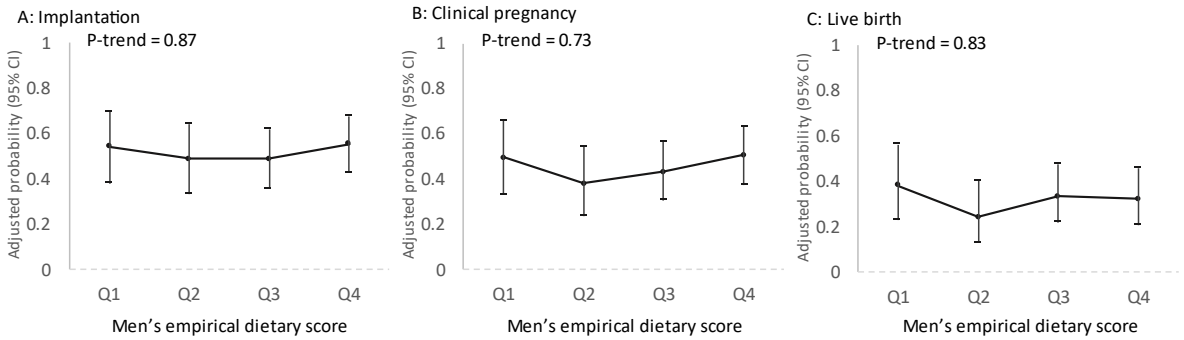
Semen parameter	Median [25th – 75th percentile]	Proportion of men below WHO reference value, %	WHO Reference value (3)
Volume (ml)	2.7[1.9-3.4]	13.3	1.5ml
Total sperm count (×10^6)	137.7[69.1-227.4]	7.8	39×10^6
Sperm concentration (×10^6/ml)	54.7[26.5-93.6]	9.7	15×10^6 / mL
Total motility (%)	45.9[27.2-61.9]	39.8	40% motile
Progressive motility (%)	25.5[15.0-36.0]	62.5	32% motile
Normal Morphology (%)	6.0[4.0-8.4]	23.8	4% normal

Supplemental Figure 1. Men's empirical dietary score in relation to fertilization rate, overall and in IVF and ICSI cycles, excluding couples with a primary diagnosis of male factor infertility. *



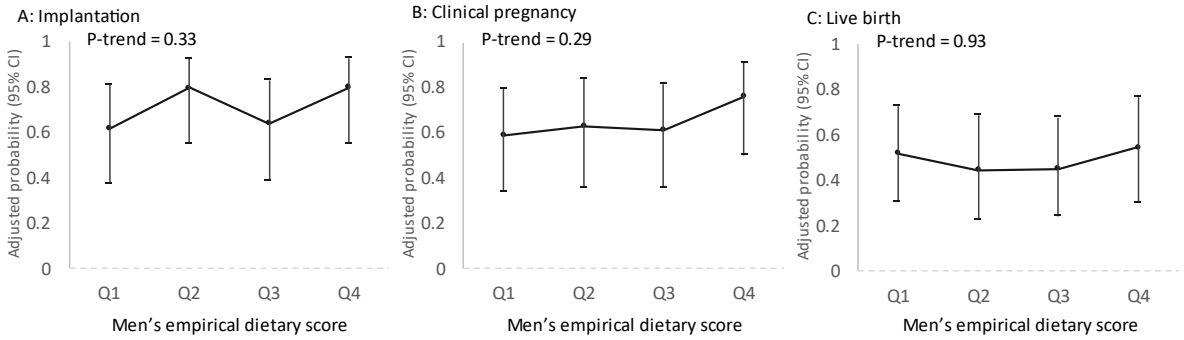
* Adjusted for men's age, women's age, men's total calories intake, total exercise, ICSI (yes/no), primary infertility diagnosis and stimulation protocol. Stratified models (B and C) do not include a term for ICSI. Q: quartile.

Supplemental Figure 2. Men's empirical dietary score in relation to clinical outcomes of infertility treatment with ART, excluding couples with a primary diagnosis of male factor infertility(N = 146 couples, 272 cycles). *



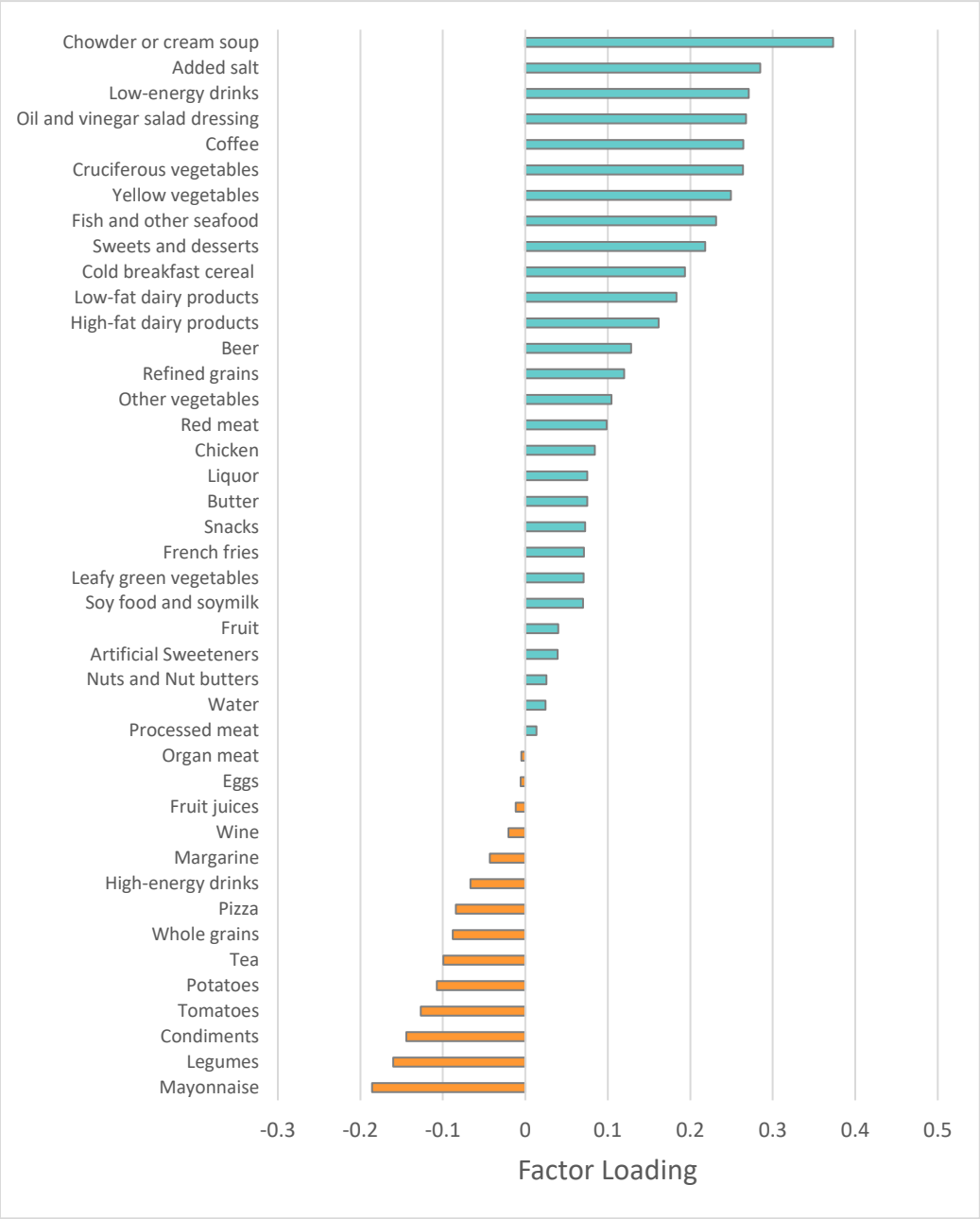
* Adjusted for men's age, women's age, men's total calories intake, total exercise, primary infertility diagnosis and stimulation protocol. Q: quartile.

Supplemental Figure 3. Men's empirical dietary score in relation to clinical outcomes of infertility treatment with ART, excluding ICSI cycles (N = 92 couples, 124 cycles).*



* Adjusted for men's age, women's age, men's total calories intake, total exercise, primary infertility diagnosis and stimulation protocol. Q: quartile.

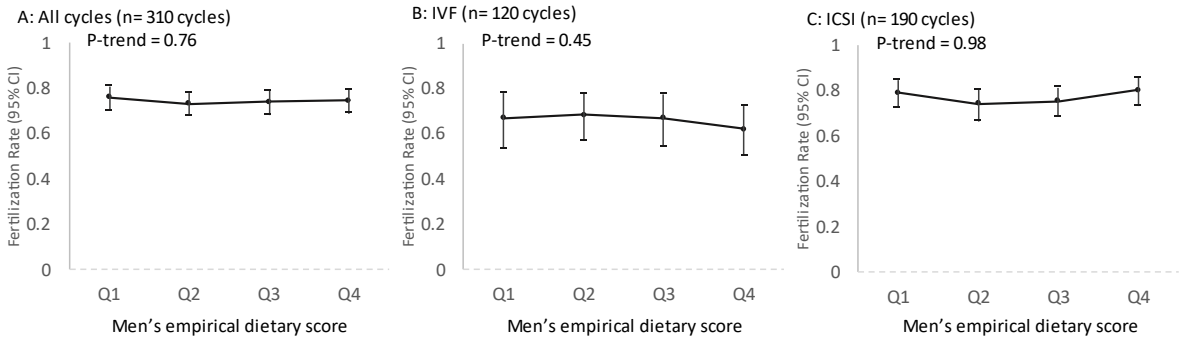
Supplemental Figure 4. Factor loadings for reduced rank regression with food groups as predictive variables and semen parameters as response variables, without adjustment for abstinence time.*



* Turquoise bar: Positive factor loading food groups.

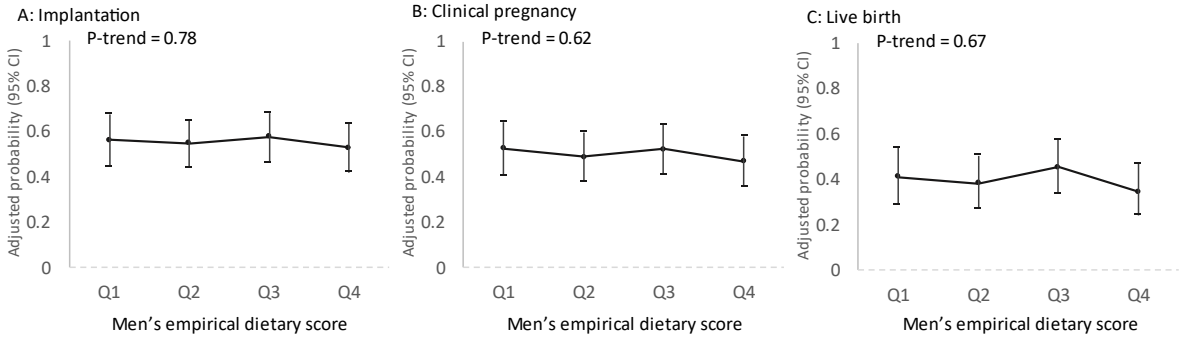
Orange bar: Negative factor loading food groups.

Supplemental Figure 5. Men's empirical dietary score (without adjustment for abstinence time) in relation to fertilization rate, overall (A) and in IVF (B) or ICSI (C).*



*Adjusted for men's age, women's age, men's total calories intake, total exercise, ICSI (yes/no), primary infertility diagnosis and stimulation protocol. Stratified models (B and C) do not include a term for ICSI. Q: quartile.

Supplemental Figure 6. Men's empirical dietary score (without adjustment for abstinence time) in relation to clinical outcomes of infertility treatment with ART.* (N = 231 couples, 406 cycles).*



*** Adjusted for men's age, women's age, men's total calories intake, total exercise, primary infertility diagnosis and stimulation protocol. Q: quartile.**

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SUMMARY OF PAPER 1 AND PAPER 2 CONCLUSIONS

Paper 1; We identified two underlying dietary patterns using PCA, which jointly explained 19.3% of the variance in food intake. Dietary Pattern One was characterized by greater intakes of processed meats, unprocessed red meats, high-fat dairy, beer, french fries, cream soups, refined grains, pizza, snacks, and sweets. On the other hand, Dietary Pattern Two was characterized by greater intakes of fruit, vegetables, legumes, soy foods, whole grains, nuts and nut butters. Despite sperm concentration being associated with one of these patterns, men's adherence to two data-derived dietary patterns was not associated with outcomes of infertility treatment with ART.

Paper 2; A dietary score capturing the overall relation of men's diet with semen quality mostly mirrors previously reported associations of diet with individual semen parameters, although there were some notable differences. Despite the empirical dietary score being associated with all standard semen parameters, the empirical dietary score was not associated with outcomes of infertility treatment with ART.

DISCUSSION AND PERSPECTIVES

The principal strength of this thesis project was using two complementary analytical approaches to characterize men's dietary patterns and their relation to a couple's infertility treatment outcomes. Paper 1 investigated the association between underlying dietary patterns using PCA and ART outcomes. To our knowledge, this is the first study to date examining the association between men's dietary patterns and couples' ART outcomes. For Paper 2, we applied RRR to identify a dietary pattern that captured the overall relation of men's diet with semen quality. RRR determines linear functions of predictors (food groups) by maximizing the explained variation in responses (all semen parameters)(19). The strength of RRR is that it eliminates the impact of any prior beliefs, knowledge, specialist advice, behavioral change due to knowing their current fertility status on how diet might impact a couple's fertility.

As we mentioned in the papers, our studies have some limitations. First, we used the data from men in couples presenting to an academic fertility center in Massachusetts. Therefore, while findings may be generalizable to other couples seeking fertility treatment, it is uncertain whether and to what extent these findings may also generalize to couples attempting conception naturally. An additional problem of studying couples in fertility centers, particularly as it relates to semen quality as a study outcome, is that by the time couples present to clinic, they may have already changed a number of behaviors considered unhealthy in hopes of improving their fertility, therefore raising concerns of reverse causation. Second, given that food intake was self-reported, some measurement error is inevitable. However, we used a validated FFQ, which previously found the relationship between diet and fertility outcomes in the same population. In addition, only one

assessment was conducted at the study baseline. In terms of identifying dietary patterns, data-driven approaches may result in patterns that exclude potentially important components. We saw this in paper 1, where the two identified patterns did not capture intake of specific foods previously related to fertility (20). Paper 2 also raises an important question for male reproductive medicine beyond the immediate findings for diet. These findings suggest that any effect men's diet (and possibly other environmental factors) may have on a couple's ability to conceive through infertility treatment with ART is completely independent of semen quality. Therefore, any clinical interventions aimed at improving men's semen quality may have little to no impact on fertility in the setting of infertility treatment.

There are also important implications for future work of these findings. First, investigating couples attempting conception without medical assistance may provide better insights into the true biological effect of men's diet on a couple's fertility. In addition, this work highlights the importance and urgency of identifying biomarkers of male fertility potential that are independent of conventional semen parameters.

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