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ORIGINAL ARTICLE

Dietary pattern characterisation among subfertile South Asian women and the impact of vitamin B12 intake on frozen embryo transfer outcomes: a cross-sectional study

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STUDY QUESTION: Is there a difference in dietary patterns among subfertile South Asian women undergoing frozen embryo transfer (FET)?

SUMMARY ANSWER: Significant regional differences in dietary pattern exist among subfertile South Asian women undergoing FET.

WHAT IS KNOWN ALREADY: Preconception consumption of certain food groups or adopting specific dietary patterns, such as the 'Mediterranean diet', and its level of adherence have been shown to enhance the odds of achieving a successful pregnancy in women undergoing ART. However, differences in geographic location, individual preference, cultural beliefs and local availability contribute to such dietary choices. There is also a predisposition to a vitamin B12 deficiency in those of South Asian ethnicity and a predominant pattern of vegetarian food intake. There is a paucity of studies analysing the type of dietary pattern followed by South Asian women, their vitamin B12 levels and the potential impact on ART treatment outcomes.

STUDY DESIGN, SIZE, DURATION: This is a cross-sectional study of 159 South Asian women aged 21–37 years, belonging to the Eastern ($n = 75$) and Southern ($n = 84$) regions of India plus Bangladesh, and undergoing a FET cycle at a tertiary level infertility clinic between February 2019 and March 2020.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women underwent dietary assessment using '24-hour dietary recall' to capture daily nutrient consumption. A 'Food Frequency Questionnaire' listing commonly consumed foods was used to record frequency of intake. The primary outcome was the characterisation of regional dietary patterns in the cohorts using principal component analysis (PCA). Secondary outcomes included association of vitamin B12 intake and serum levels with clinical and ongoing pregnancy.

MAIN RESULTS AND THE ROLE OF CHANCE: Four components contributing to overall variance in dietary pattern were identified, namely: meat, poultry and seafood; green leafy vegetables and root tubers; fruits, dairy and sugar; nuts and oilseeds. PCA analysis showed a significantly higher consumption of two components in the East—meat, poultry and fish ($P < 0.001$); green leafy vegetables and root tubers ($P < 0.001$). All women reported taking preconception oral folic acid supplementation. The dietary intake of vitamin B12 and serum concentration correlated, showing a good validity of measured dietary intake ($r = 0.398$; $P \leq 0.001$). Compared to the Southern region, participants from the East showed a higher daily median intake of vitamin B12 (1.11 versus 0.28 mcg, respectively; $P < 0.001$) and a higher serum vitamin B12 levels (441 versus 239 pg/ml, respectively; $P < 0.001$). Ongoing pregnancy showed no association with dietary vitamin B12 intake (relative risk 0.90; 95% CI, 0.68 to 1.19) or serum vitamin B12 levels (relative risk 0.99; 95% CI, 0.73 to 1.33) after adjustments for female age, body mass index (BMI) and geographic differences. Women belonging to different quartiles of serum vitamin B12 concentration had a similar likelihood of ongoing pregnancy.

LIMITATIONS, REASONS FOR CAUTION: Self-reported dietary assessment is prone to measurement errors owing to its subjective nature and recall bias. The study was not adequately powered to detect the impact of geographic differences in vitamin B12 intake and serum levels on FET treatment outcomes, the second objective. We adjusted for potential confounders, such as female age and BMI, but it is possible that residual confounders, such as physical activity, stress and use of dietary supplements, may have influenced the results. Extrapolation of the study findings to women undergoing ART in other populations should be made with caution.

WIDER IMPLICATIONS OF THE FINDINGS: Our study findings suggest important differences in local dietary patterns within the South Asian region. Hence a personalised approach to dietary assessment and intervention when undergoing ART based on population dynamics is warranted. The geographic differences in the vitamin B12 intake or serum levels did not have an impact on the FET outcomes. There is also a need to further investigate the impact of such dietary differences on ART treatment outcomes in a large study population.

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WHAT DOES THIS MEAN FOR PATIENTS?

The consumption of certain foods or following specific diet patterns, such as the 'Mediterranean diet,' has been shown to increase chances of success following IVF treatment. However, differences in the geography, individual preference, cultural beliefs and local availability influence such dietary choices. For women with a South Asian ethnic background and who follow a vegetarian diet, variations in the vitamin B12 profile have been identified. There are very few studies assessing the dietary patterns followed by South Asian women, their vitamin B12 levels and the potential impact on IVF outcomes. This study included 159 women belonging to the Eastern and Southern regions of South Asia and undergoing a transfer of frozen embryos. The results showed a significantly higher intake of meat, poultry, fish, green leafy vegetables and root tubers by women in the East. Compared to the Southern region, participants from the East had a higher daily intake and blood levels of vitamin B12; however, these geographic variations in vitamin B12 levels did not affect their chances of becoming pregnant following IVF. Our findings highlight important differences in dietary patterns in different regions of South Asia. Hence, assessment of dietary intake and potential modifications requires a tailored approach based on the target population.

Introduction

Subfertility affects one in six couples in the reproductive age group globally (Farquhar *et al.*, 2019). Worldwide, the median prevalence of subfertility is estimated to be approximately 9% (Boivin *et al.*, 2007). A global demographic study by the World Health Organization (WHO), however, identified a higher prevalence of infertility in certain developing regions of the world, including South Asia (Mascarenhas *et al.*, 2012). Rapid urbanisation in South Asia has resulted in a significant lifestyle transition including unhealthy dietary habits and impaired physical activity (Schmidhuber and Shetty, 2005). Studies have shown a negative impact of these modifications on female fertility (Chavarro *et al.*, 2007; Anderson *et al.*, 2010; Gormack *et al.*, 2015). The number of ART cycles being performed worldwide is steadily increasing (Adamson *et al.*, 2018). Therefore, in view of changing dietary habits, it is important to delineate the impact of nutrition on ART outcomes in different subpopulations across the world.

Over the past few decades, nutrition research has evolved from single nutrient studies focussing on specific components, such as vitamins, dietary sugars and fat, to those analysing dietary patterns that capture complex interactions of different food constituents consumed on a routine basis and the level of adherence (Mozaffarian *et al.*, 2018). Different dietary patterns have been identified globally namely Nordic, Mediterranean and the Western diet, each characterised by varied nutrient sources constituting the 'food pyramid' and showing inherent health benefits and adverse effects (Raits and Kirse-Ozolina, 2019). A Dutch study recruited women undergoing ART and estimated their adherence to Dutch dietary recommendations by calculating the Preconception Dietary Risk score (PDR) and found a positive association between high adherence and on-going pregnancy rates (Twigt *et al.*, 2012). Two studies from Europe, by Vujkovic *et al.* (2010) and Karayiannis *et al.* (2018), reported that a greater adherence to a 'Mediterranean diet' pattern (rich in vegetable oils, fish, legumes, whole grains and low in meat) preconception, enhanced the odds of achieving a successful pregnancy through ART. Further to this, a study group from the USA classified 357 women undergoing ART into quartiles based on adherence to four different dietary patterns—MedDiet, Fertility diet, Alternate Healthy Eating Index 2010 and profertility diet (Gaskins *et al.*, 2019). Contrary to findings from the European studies, they showed no additional improvement in live birth rate above the second quartile of adherence to the MedDiet. The extrapolation of dietary study findings beyond the study population is, therefore, debatable, because differences in the geographic location, individual preference, cultural beliefs and local availability can contribute to or dictate dietary choices.

In recent years, the role of Vitamin B12 in influencing ART outcomes has generated much interest. Vitamin B12 is a critical cofactor of the 1-carbon pathway that supports vital processes such as DNA, protein and phospholipid biosynthesis (Stipanuk, 2004). Periconception derangements in the pathway have been attributed to poor reproductive outcomes, such as implantation failure, miscarriage and placental dysfunction during pregnancy (Steegers-Theunissen *et al.*, 2013). South Asian ethnicity and a predominant pattern of vegetarian food consumption have been associated with an increased prevalence of vitamin B12 deficiency (Gammon *et al.*, 2012). A systematic review of dietary patterns in India identified most patterns to be predominantly vegetarian, consisting of fruits, vegetables and pulses with animal protein consumption to be more frequent in the Eastern region (Green *et al.*, 2016). A study from the USA reported women with higher pre-IVF serum vitamin B12 levels to have twice the probability of live birth compared to those with lower values (Gaskins *et al.*, 2015). However, any impact in South Asian women, who are prone to vitamin B12 deficiency, is yet to be ascertained.

Information on the dietary patterns prevailing in South Asian women is notably lacking, thereby limiting meaningful conclusions on its impact on ART. We planned a study to characterise the dietary patterns in two geographically diverse regions in South Asia and to estimate potential differences in dietary nutrient intake between them. We also investigated the impact of intake of vitamin B12 on frozen embryo transfer (FET) treatment outcomes.

Materials and methods

Study population

We carried out a cross-sectional study between February 2019 and March 2020 at Christian Medical College, Vellore, India, which is a tertiary level infertility clinic, frequented by couples from the Eastern and Southern regions of South Asia. The primary objective of the study was characterisation of dietary patterns prevalent in South Asian women who were planned for FET treatment and the cross-sectional design was planned to examine the same. The second objective was to explore any association between vitamin B12 intake and FET outcomes and a cohort design was used to investigate the same. We invited subfertile women who were scheduled to undergo FET to participate in the study. Participants aged less than 37 years, having a body mass index (BMI) less than 35 kg/m^2 and undergoing FET in an HRT cycle were included in the study. Exclusion criteria included a history of diabetes mellitus, familial hypercholesterolaemia or intestinal disorders requiring diet modification. Of 348 women who underwent FET during this period, 159 women who met the eligibility criteria and provided a written informed consent were enrolled. They were recruited only once in the study. Women were broadly categorised into two geographic cohorts based on domicile—Southern region (Indian states of Tamil Nadu, Andhra Pradesh, Karnataka, Kerala and Telangana) and Eastern region (Indian states of West Bengal, Jharkhand, Bihar, Assam and Tripura; country of Bangladesh) of South Asia. The study protocol was approved by the Institution Review Board and carried out in accordance with the Helsinki declaration.

Endometrial preparation for FET and enrolment

All frozen-thawed embryos were transferred in an HRT cycle. An escalating dose of oral oestrogen (Oestradiol valerate, Progynova, Bayer, Germany) 2–6 mg daily, was used for endometrial preparation. After 14 days of initial estrogen priming, a transvaginal ultrasonography (TVUS) (GE Voluson, probe frequency 7.5 Hz) was performed on Day 15 to measure the endometrial thickness and exclude follicular development. If the endometrial thickness was >7 mm, vaginal natural micronised progesterone (Naturogest, Zydus Cadila, India) was commenced at a dosage of 400 mg twice daily. The timing of embryo thawing and transfer was planned according to the developmental stage at cryopreservation. Eligible women who were willing to participate were included in the study at this stage after signing a written informed consent.

Information regarding demography, type of infertility, and current treatment details, such as number of FET attempts, embryo grade, number of embryos transferred, were recorded and a detailed face-to-face dietary assessment was performed by trained staff from the Department of Dietetics.

FET and luteal support

All the embryos transferred had been cryopreserved using vitrification. FET was performed either on the same day as, or the day after, thaw. Between one and three embryos at the cleavage stage or one to two embryos at the blastocyst stage were transferred, according to department policy. Following FET, luteal support was continued with oral oestradiol valerate 2 mg three times in a day and vaginal micronised progesterone 400 mg twice daily. Serum βhCG assessment was performed 14 days after FET. A value exceeding 5 mIU/ml was defined as a positive pregnancy. A follow-up TVUS was performed 2 weeks later to confirm clinical pregnancy. Luteal support was continued until 10 weeks of gestation.

Dietary assessment

Using the '24-hour dietary recall' method, participants were asked to recollect the foods routinely consumed over 24 hours including breakfast, mid-morning, lunch, evening snacks, dinner, and late night. Based on the food item consumed, the quantity and nutritive value, the actual measure of calories (in kilocalories, kcal), carbohydrates (in grams, g), fats (in grams, g), proteins (in grams, g), omega-3 polyunsaturated fatty acid (PUFA, in milligrams, mg) and vitamin B12 (in micrograms, mcg) was estimated. The nutritive value was compiled from the food composition tables validated and published by the National Institute of Nutrition (NIN), Hyderabad, India (Longvah *et al.*, 2017). During the same interview, a 'Food Frequency Questionnaire' (FFQ), listing around 50 food items commonly consumed by the study population was administered to record the frequency of intake of each item (never, daily, weekly or monthly) over the previous 1 month. The individual food items were consolidated into 12 major food groups—cereals and millets; pulses and legumes; green leafy vegetables; roots and tubers; other vegetables; fats and oil; fish and sea food; meat and poultry; milk and milk products; sugar and jiggery (traditional cane sugar); condiments and spices; nuts and oil seeds. Blood samples were drawn for estimating the serum vitamin B12 levels on the same day as administering the dietary questionnaire. Serum vitamin B12 was measured by an electrochemiluminescence immunoassay technique on the Cobas E immunoassay analyser (Roche Diagnostics, Switzerland). Normal serum vitamin B12 level was defined as 200–950 pg/ml. The lowest detection cut-off for the assay was 50 pg/ml.

Outcomes

The primary outcome measure was characterisation of the dietary patterns prevalent in South Asian women belonging to the Eastern and Southern regions. Secondary outcomes included geographic differences in dietary intake of calories, protein, fat, vitamin B12, omega-3 PUFA and serum levels of vitamin B12. The association between serum vitamin B12 concentration and clinical/ongoing pregnancy were also recorded as a secondary outcome. Clinical pregnancy was defined as a pregnancy diagnosed by ultrasonographic visualisation of one or more gestational sacs. Ongoing pregnancy was defined as a viable intrauterine pregnancy of ≥12 weeks confirmed on TVUS (Braakhekke *et al.*, 2014).

Statistical methods

Data were summarised using mean ± SD/median (inter quartile range, IQR) for continuous variables, depending on normality. Categorical variables were presented as a frequency and percentage. Independent Student's *t*-test and chi-square test were used for region-wise comparison of continuous and categorical variables, respectively. Skewed variables were log-transformed and used for all further analysis. The effect size for the FET outcomes was presented as relative risk (RR) and 95% CI, using a generalised linear model with log link. For dietary pattern analysis, principal component analysis (PCA) with varimax rotation was used for factor extraction of food groups. Assumption checks for normality of data set were done using p-p plot and multivariate sampling adequacy was verified using the Kaiser–Meyer–Olkin (KMO) test. A KMO measure of more than 0.50 was considered acceptable. The homogeneity of variance was tested using the Bartlett test of sphericity and accepted when $P < 0.05$. Components with an Eigenvalue greater than one, were retained and the percentage of variation explained by the extracted components was determined. Component scores were then assigned for each component to compare the consumption between the two geographic cohorts. Pearson's correlation coefficient was applied for measuring the dependence between serum levels and dietary intake of vitamin B12. Women were categorised into quartiles (Q) based on the serum vitamin B12 levels and results presented as RR (95% CI) comparing Q2, 3, and 4 with Q1 (reference group). All the analyses were performed using STATA/IC 16.0 (StataCorp, 4905 Lake Drive, College Station, TX 77845, USA). A value of $P < 0.05$ was considered statistically significant.

Results

Of the 159 women satisfying the inclusion criteria, 158 women underwent FET. Embryo transfer was deferred in one woman due to personal reasons and hence excluded from assessment. A total of 75 women belonged to the eastern region and 83 belonged to the South. The baseline clinical characteristics of the study participants based on geographic distribution are summarised in Table I. No statistically significant difference was observed when mean female age and BMI, type and cause of infertility of women, presence of PCOS, FET cycle number and day of embryo transfer were compared between the two regions. Ongoing pregnancy and clinical pregnancy rates were comparable between the Eastern and Southern regions. Table I also presents the daily intake of macronutrients and micronutrients in the two geographic regions. Compared to the Southern region, participants from the East showed a higher mean daily energy intake (1810.20 versus 1637.66 kcal, respectively; $P = 0.002$) and protein (61.68 versus 48.31 gm, respectively; $P < 0.001$). With micronutrients, women from the East recorded a higher daily consumption of vitamin B12 (1.46 versus 0.35 mcg, respectively; $P < 0.001$) compared to the South. Median serum vitamin B12 levels were also higher in women from the East compared to the South (440.5 versus 249.0, respectively; $P < 0.001$). In contrast, omega-3 PUFA intake was more prominent in the South than the East (246 versus 166 mg; $P < 0.001$). All the subjects reported taking preconception oral folic acid supplementation (single dose of 5 mg once daily). None of the study participants reported a smoking habit or alcohol consumption.

Dietary patterns were characterised by performing factor extraction for eight food groups using PCA with varimax rotation. Four components were identified with Eigen values greater than one and together they accounted for 65.9% of the total variance in the consumption pattern. Factor loadings for the eight food groups after rotation are presented in Table II. Factor loadings more than 0.5 were included. The first component, accounting for 22% of the total variance in food consumption pattern, had a high factor loading for meat, poultry, fish and other seafood (protein rich, non-vegetarian food). The second component was characterised by intake of green-leafy vegetables, roots and tubers (plant-based foods) and represented 18.1% of total variance. The third component had high factor loadings on dairy and sugars, whereas the fourth component was loaded by a high intake of fruits, nuts and oilseeds, which are rich in micronutrients. The third and fourth components explained 13.3% and 12.5% of the total variance in dietary pattern, respectively. For each of the four PCA-derived components, we calculated individual component scores and compared score differences between women from the Eastern and Southern regions (Table III). A higher consumption of protein-rich, non-vegetarian food and plant-based foods was observed in the Eastern region compared to the South and this was found to be statistically significant. A trend towards higher dairy and sugar consumption was observed in the South compared to east but did not reach statistical significance.

We, subsequently, analysed the association between vitamin B12 and FET treatment outcomes (Table IV). Considering the geographic differences, we did a subgroup regression of Eastern and Southern regions, separately. With both the cohorts, the analysis showed no association between dietary vitamin B12 intake or serum levels and clinical or ongoing pregnancy rates, depicted by similar RR estimates. These findings were further corroborated when the regression was performed on the whole study population by including the interaction effect of vitamin B12 and geography, which showed no association between vitamin B12 and the outcomes. Additionally, 152 women were grouped into quartiles (Q) based on serum vitamin B12 levels and their association with clinical and ongoing pregnancy was expressed as RR and 95% CI by comparing Q2, Q3 and Q4 with Q1 (reference) after adjusting for age, BMI and geography (Table V). The probability of

Table 1 Clinical and dietary characteristics among South Asian women undergoing ART, based on geographic location.

	Geography		*P*-value
	East	South	
N	**75**	**83**	
Clinical characteristics			
Age*	30.80 + 4.28	30.63 + 3.74	0.786
BMI (kg/m^2) *	24.49 + 3.65	25.31 + 3.68	0.162
PCOS, n (%) #			0.145
Yes	26 (34.7)	39 (47.0)	
No	49 (65.3)	44 (53.0)	
Type of infertility, n (%) #	0.315		
Primary	53 (70.7)	52 (62.7)	
Secondary	22 (29.3)	31 (37.3)	
Cause of infertility, n (%) #			0.158
Anovulation	20 (26.7)	32 (38.6)	
Male	17 (22.7)	21 (25.3)	
Tubal	12 (16)	13 (15.7)	
Unexplained	5 (6.7)	8 (9.6)	
Endometriosis	18 (24)	8 (9.6)	
Combination	2 (2.7)	1 (1.2)	
DOR	1 (1.3)	–	
FET cycle number, n (%) #			0.275
1	63 (84)	61 (73.5)	
2	9 (12)	18 (21.7)	
3	3 (4)	3 (3.6)	
4	–	1 (1.2)	
Day of embryo transfer, n (%) #			0.341
Cleavage stage	26 (34.7)	30 (36.1)	
Blastocyst	49 (65.3)	53 (63.9)	
Number of embryos transferred, n (%) #			0.130
1	9 (12)	14 (16.9)	
2	65 (86.7)	63 (75.9)	
3	1 (1.3)	6 (7.2)	
Dietary characteristics*			
Energy intake (Kcal/day)*	1810.20 + 389.04	1637.66 + 304.52	0.002
CHO intake (gm/day)	268.41 + 87.42	260.20 + 66.56	0.505
Protein intake (gm/day)*	61.68 + 17.76	48.31 + 13.04	<0.001
Fat intake (gm/day)	45.87 + 16.90	42.23 + 14.93	0.151
ω3 PUFA intake (mg/day)**	166 (115, 218)	246 (196, 311)	<0.001
Vitamin B12 intake (mcg/day)**	1.46 (0.28, 2.90)	0.35 (0.14, 0.70)	<0.001
Serum vitamin B12 (pg/ml)**	440.5 (335, 618)	249 (164, 325)	<0.001
ART outcomes, n (%) #			
Positive Pregnancy	46 (61.3)	48 (57.8)	0.654
Clinical pregnancy	40 (53.3)	43 (51.8)	0.848
Ongoing pregnancy	29 (38.7)	34 (41.0)	0.768

Values are presented as mean ± SD or median (interquartile range) for age, BMI and dietary characteristics.
*Mean ± SD presented and significance assessed by Student's *t*-test.
#n (%) presented and significance tested by Pearson's Chi-square.
**Median (IQR) presented. Followed by a log transformation, significance was assessed using Student's *t*-test.
CHO, carbohydrate; DOR, diminished ovarian reserve; FET, frozen embryo transfer; PCOS, polycystic ovarian syndrome; ω3 PUFA, omega 3 polyunsaturated fatty acid.

Table II Principal component analysis showing factor loading (>0.5) of each food group with the four dietary components.

Food groups	Component 1	Component 2	Component 3	Component 4
Green leafy vegetables		0.7340		
Roots and tubers		0.5380		
Nuts and oils				0.5233
Fruits				0.7535
Fish and seafood	0.6194			
Meat and poultry	0.5939			
Milk and milk products			0.6133	
Sugar and jaggery*			0.7829	

Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy = 0.540; Bartlett's test of sphericity for food groups ($P < 0.001$).
Extraction method: Principal component analysis; Rotation method: Varimax.
*Traditional cane sugar.

Table III Comparison of component scores between the Eastern and Southern regions for the four PCA-derived components.

Components	Component score based on geography		*P*-value
	East (n = 75)	South (n = 83)	
Protein rich foods	0.83 (1.13)	−0.72 (0.83)	<0.001*
Plant based foods	0.26 (1.14)	−0.23 (1.10)	0.006*
Dairy and sugars	−0.11 (1.01)	0.08 (1.20)	0.288
Fruits, nuts and oilseeds	0.03 (0.97)	−0.02 (1.18)	0.764

Values presented as mean (SD) and significance assessed using Student's *t*-test.
*Statistically significant ($P < 0.05$).
PCA, principal component analysis.

Table IV Impact of dietary B12 intake and serum vitamin B12 levels on frozen embryo transfer treatment outcomes.

	Clinical pregnancy		Ongoing pregnancy	
	RR (95% CI)	*P*	RR (95% CI)	*P*
Vitamin B12 intake (mg/d)*				
East	0.99 (0.71, 1.38)	0.940	0.91 (0.63, 1.32)	0.618
South	0.84 (0.56, 1.28)	0.422	0.88 (0.57, 1.38)	0.587
Serum B12 (pg/ml)*				
East	0.87 (0.62, 1.23)	0.443	1.02 (0.66, 1.59)	0.919
South	1.02 (0.70, 1.48)	0.928	0.98 (0.65, 1.48)	0.914
Interaction between vitamin B12 intake and geography	0.95 (0.76, 1.20)	0.679	0.90 (0.68, 1.19)	0.461
Interaction between serum B12 level and geography	0.97 (0.78, 1.22)	0.820	0.99 (0.73, 1.33)	0.930

*For each variable a separate regression equation was modelled as per geography.
Note: Relative risk estimates calculated after adjustments for age and BMI.
RR, relative risk.

clinical and ongoing pregnancy was similar across quartiles of serum vitamin B12 concentration. The dietary intake of vitamin B12 and serum concentration correlated, showing a good validity of measured dietary intake ($r = 0.398$; $P \leq 0.001$).

Discussion

The current study identified important differences in the dietary pattern among subfertile women belonging to two geographically distinct regions of South Asia. Intake of protein-rich non-vegetarian and plant-

Table V **Association between serum vitamin B12 levels across quartiles and ART outcomes.**

Serum vitamin B12 Quartiles* (pg/ml)	N = 152	Clinical pregnancy		Ongoing pregnancy	
		Relative risk (95% CI)	*P*	Relative risk (95% CI)	*P*
Q1 (50–228)	38	1.00 (reference)	–	1.00 (reference)	–
Q2 (229–329)	38	1.11 (0.60, 2.05)	0.732	0.93 (0.46, 1.86)	0.829
Q3 (330–453)	38	0.83 (0.41, 1.66)	0.591	0.74 (0.33, 1.63)	0.450
Q4 (454–1246)	38	0.82 (0.38, 1.76)	0.613	0.79 (0.34, 1.86)	0.589

*Quartile values presented as (minimum-maximum); Relative risk adjusted for age, BMI and geography.

based food groups were more frequent in the cohort of subfertile women from the East compared to South. Lower serum vitamin B12 levels were observed in the Southern cohort compared to Eastern cohort. However, the geographic differences in the vitamin B12 intake or serum levels did not have an impact on the FET outcomes.

A systematic review by Green *et al.* (2016), which focussed on dietary patterns in India, identified wide regional variations. The authors reported a high prevalence of animal product (meat and fish) consumption in the Eastern population. These findings are in agreement with the current study findings where the first component characterised by 'protein-rich non-vegetarian' food intake was higher among the Eastern cohort. 'Plant-based foods', rich in antioxidants, vitamins and minerals, constituted the second component and was again found to be more predominant in the Eastern region than the Southern one. This combination of higher animal protein and vegetable intake in the East closely reflects the results on 'diet mixing' described by Padmadas *et al.* (2006) using Indian data collected under the National Family Health Survey-2 (NFHS-2), focussing on individual dietary information on daily, weekly and occasional intake of selected food groups from married Indian women. They classified women into 'diet mixing clusters' based on the degree of combined vegetarian and non-vegetarian food intake. A 'high' diet mixing pattern, characterised by consumption of vegetables and pulses/beans on a daily basis along with animal produce on a weekly basis, was more prevalent in the East compared to the Southern region. Women in the South had a greater leaning towards the third component represented by intake of dairy and sugars, including jaggery. A similar observation for high dairy product intake was noted by Padmadas *et al.* (2006) in their cross-sectional survey in the Southern population compared to East. The fourth component, including fruits, nuts and oilseeds, which are rich sources of fatty acids, vitamins and minerals, was comparable between women of the two regions. These study results reinforce existing evidence on variations in dietary patterns among the subpopulations in South Asia and lends credibility to the argument against attempts at homogenisation for dietary assessment and intervention.

We also analysed the dynamics of vitamin B12 consumption in the study population due to growing interest in its role in implantation and the increased prevalence of B12 deficiency in women belonging to the South Asian ethnicity and following a predominantly vegetarian diet (Boxmeer *et al.*, 2009; Gammon *et al.*, 2012; Rizzo *et al.*, 2016). In the East, a higher intake of non-vegetarian foods, which are rich sources of vitamin B12, possibly explains the higher median daily intake of vitamin B12 when compared to the South. Serum vitamin B12 levels positively correlated with the dietary intake. However, despite significant differences in dietary intake and serum B12 levels between the two geographic cohorts, there was no significant impact of this finding on the ongoing and clinical pregnancy rates. Also, the ongoing and clinical pregnancy rates among women belonging to different quartiles of serum vitamin B12 concentration were similar. This finding is in agreement with the results of a prospective cohort study from Scotland involving 602 women undergoing IVF, which reported on the effect of vitamin B12 (Haggarty *et al.*, 2006). The authors found no significant association between dietary intake or plasma vitamin B12 levels and live birth after IVF. But the evidence remains conflicting. An American study by the EARTH study group analysed serum vitamin B12 levels in 100 subfertile women who underwent 154 ART cycles (Gaskins *et al.*, 2015). They reported the probability of live birth in the highest quartile of serum vitamin B12 to be 2.04 (95% CI: 1.14, 3.62) times that of the lowest quartile. However, this association was not evident in the intermediate quartiles. However, the study was limited by the number of participants who were truly vitamin B12 deficient to assess the real impact and many participants received multivitamin supplementation that could have influenced vitamin B12 levels and the results.

To date, this is one of the foremost studies focussing on dietary patterns in South Asian women undergoing assisted reproduction. We identified the dietary pattern using an *a posteriori* approach, which captures the interaction between various food groups that generally constitute a habitual diet and assigned factor scores to identify differences in dietary patterns in Eastern and Southern regions. However, the drawback of such an approach is the questionable utility of derived estimates to predict the impact on treatment outcomes. Employing diet scores using *a priori* knowledge can help to circumvent this limitation (Panagiotakos, 2008). However, due to paucity of evidence on prevailing dietary patterns in South Asian women, it was challenging to adopt such a model in our study upfront.

The study has a few limitations to address. The usual dietary intake was estimated using a 24-hour dietary recall method. Although this method of assessment captures a diverse group of food groups consumed and their quantity, measurement errors are possible because of its subjective nature and risk of recall bias. The FFQ used in the study did not capture the portion size, which could have facilitated validation of 24-hour dietary intake estimates. The study was not adequately powered to detect the impact of geographic differences in vitamin B12 intake and serum levels on FET treatment outcomes, which was the second objective. It is likely that a small sample size precluded a more robust estimation of effect size. This was, however, an additional analysis based on our primary outcomes from dietary characterisation among the two geographic cohorts, which identified a greater consumption of protein-rich non-vegetarian food in the Eastern region (an essential source of vitamin B12) and a significant difference in serum levels of vitamin B12. Secondly, our analysis was restricted to women undergoing FET. The ideal scenario would be to assess the impact of diet on the entire ART cycle and report outcomes related to different IVF processes (Noli *et al.*, 2020). However, we planned to study the impact of vitamin B12 mainly on the implantation process and the frozen cycle model provided a more homogenous cohort of women with good prognosis. The inclusion of FET cycles alone, helped minimise the impact of potential confounders associated with fresh ART cycles, such as differential response to ovarian stimulation and resultant differences in oocyte yield, which has a major influence on treatment outcomes (Sunkara *et al.*, 2011). The detrimental effect of supraphysiological levels of hormones on the endometrium and its receptivity is also well established in fresh cycles (Bourgain and Devroey, 2003). With planned FET cycles, the attrition was almost nil, as all the recruited patients underwent embryo transfer. While deriving outcome measures, we adjusted for potential confounders such as female age and BMI. Nevertheless, it is possible that residual confounders, such as physical activity, stress and use of dietary supplements, may have influenced the results.

Conclusion

In conclusion, significant variations in the dietary pattern were characterised among different geographic cohorts of subfertile South Asian women undergoing ART. A lower vitamin B12 intake and serum level noted in the Southern cohort likely reflects a dietary behaviour influenced by geocultural preferences. However, we did not find an impact of low serum vitamin B12 levels on FET treatment outcomes, possibly owing to smaller sample size. The study clearly underscores the need for a population-specific dietary assessment and intervention rather than a 'one size fits all' approach. Overall, the study findings can be used to design more robust prospective studies using advanced nutritional assessment tools to further elucidate population-specific dietary behaviour, the adherence to such patterns and their impact across ART treatment outcomes.

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Authors' roles

M.S.K. conceived the idea for the project, which was executed by A.R. with assistance from M.S.K., A.T.K. and M.K. M.B. performed the dietary assessment. A.R. and M.G. performed the data analysis. A.R. wrote the manuscript with intellectual input from M.S.K. and A.T.K. All authors critically appraised the manuscript and accept responsibility for the contents of the final manuscript for publication.

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Conflict of interest

The authors have nothing to disclose.

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