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Elucidating the chrononutrition patterns and sleep quality among subfertile patients with different vitamin D levels

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Subfertility affects about 10–15% of Malaysians, characterized by difficulty conceiving after 12 months of unprotected intercourse. Emerging research suggests that vitamin D deficiency, influenced by dietary intake and sleep patterns, may contribute to fertility. This study examines the relationship between chrononutrition patterns, sleep quality and vitamin D deficiency in subfertile patients. A cross-sectional study was conducted at two hospitals in Selangor, Malaysia, involving 140 subfertile individuals who fit the inclusion and exclusion criteria. Data were collected through self-administered questionnaires, including food frequency questionnaires (FFQ), the Chrononutrition Profile Questionnaire (CPQ), and the Pittsburgh Sleep Quality Index (PSQI). Serum vitamin D levels were measured through 25-hydroxyvitamin D blood test. Statistical analyses were performed using SPSS version 29. Results revealed a significant difference in mean serum vitamin D levels between male and female participants ($p < 0.001$), with 76.7% of the females being vitamin D deficient. Most parameters were not significant in males compared to females. Notably, a negative correlation was found between vitamin D levels and both energy and fat intake in females ($p < 0.05$). Female gender was identified as a significant determinant of vitamin D deficiency (OR 5.186, $p < 0.001$), while poor evening eating habits were strongly associated with deficiency (AOR 10.553, $p < 0.05$). These findings highlight the importance of gender and dietary patterns in vitamin D deficiency among subfertile patients. Hence, there is a need for targeted nutritional and lifestyle interventions.

Keywords Vitamin D deficiency, Dietary intake, Chorononurition, Sleep quality, Subfertility

Subfertility is defined as the inability to conceive after one year of regular unprotected intercourse. Specifically, primary subfertility refers to those who never achieved pregnancy, whereas secondary subfertility refers to those who previously achieved pregnancy but were unable to achieve subsequent pregnancy[1,2]. This is not an absolute condition, and its definition distinctively indicates the probability of conception with time. The initial evaluation of subfertility involves confirming ovulation, a normal uterine cavity, patent fallopian tubes in women, and normal semen parameters in men[3]. The primary assessment will be followed by evaluations of other blood parameters and lifestyle factors. All modifiable factors will be considered to improve the effectiveness of intervention in this group. However, for women diagnosed with unexplained fertility issues, IVF treatment was found to be the best option after two years of trying to conceive[3].

There is increasing interest in the potential relationship between vitamin D levels with fertility issues in both genders. Emerging evidence suggests gender-specific differences in vitamin D metabolism, which may be influenced by factors such as hormonal variations, differences in body composition, and lifestyle behaviors. In

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women, estrogen enhances vitamin D activation by increasing the expression of vitamin D receptors (VDR) in tissues such as the intestines, bones, and kidneys. It also promotes the conversion of vitamin D into its active form, 1,25-dihydroxyvitamin D3[4]. In men, testosterone levels interact with vitamin D by influencing the expression of VDR in the testes[4].

Furthermore, lifestyle differences, such as dietary habits, sun exposure, and physical activity, often vary between genders and can influence vitamin D levels. Women generally have a lower dietary intake of vitamin D compared to men, partly due to differences in food preferences and dietary habits[5]. Additionally, cultural and societal norms may lead to variations in clothing styles, affecting sun exposure. Women are more likely to use sunscreen and wear clothing that covers most of their skin, reducing their sun exposure compared to men[5,6]. Physical activity levels also differ, with men typically engaging in more outdoor activities, which can increase sun exposure and subsequently vitamin D synthesis[5]. Due to these lifestyle behaviors and hormonal differences, a gender difference in vitamin D deficiency has been observed in subfertile individuals, with women often having higher deficiency rates than men[5–7].

Recent studies also indicate that sufficient vitamin D levels may significantly influence reproductive health. In women, vitamin D is found to affect ovarian function, oocyte quality, and implantation efficacy. Some studies have established the correlations between low levels of vitamin D levels and conditions such as polycystic ovarian syndrome (PCOS) and endometriosis (Matevossian et al. 2019), which may affect fertility[8,9]. For instance, vitamin D deficiency has been linked to insulin resistance and hormonal imbalances, which are common in PCOS and can impair fertility[10]. Additionally, vitamin D receptors are present in the ovaries and uterus, suggesting their role in regulating reproductive functions such as egg quality and implantation[10].

In men, vitamin D has an important role in sperm production and quality. Low levels of vitamins have been associated with decreased sperm motility and increased DNA fragmentation in sperm cells[11]. Vitamin D regulates calcium ion channels, specifically CatSper channels, which are crucial for the flagellar movement that allows sperm to swim toward the egg[12]. Additionally, vitamin D supports mitochondrial energy production in sperm cells, further enhance the sperm motility[13]. Vitamin D also has antioxidant properties that protect sperm DNA from oxidative stress caused by reactive oxygen species (ROS)[14]. Deficiency in vitamin D impairs these functions, resulting in reduced motility and increased DNA damage[14].

Consequently, consultants are progressively taking vitamin D levels into account when assessing and addressing reproductive concerns[15,16]. Due to the heterogeneity of results from the growing body of research, it is necessary to comprehensively elucidate the mechanisms involved and to ascertain whether vitamin D supplementation can improve fertility[17,18].

Vitamin D deficiency has been increasingly recognized as a potential factor influencing reproductive health, yet its specific impact on subfertile populations remains underexplored, particularly within the Malaysian healthcare context. Despite evidence suggesting that sufficient vitamin D levels are associated with improved fertility outcomes, findings have been inconsistent, especially regarding the roles of dietary patterns, chrononutrition, and sleep quality. Identifying these gaps is crucial, as these factors are associated with vitamin D metabolism. Therefore, more empirical evidence is needed to provide insights for managing subfertility. Furthermore, understanding gender differences in vitamin D status and its determinants is essential, given the distinct hormonal and lifestyle factors between men and women that may influence reproductive health[19].

The relationship between dietary intake, chrononutrition, and sleep quality is complex and potentially influences vitamin D levels. There is a bidirectional effect between the circadian rhythm that controls sleep patterns and nutrient metabolism[20]. A well-balanced diet that aligns with the natural circadian rhythm can promote better sleep quality. Conversely, irregular eating patterns or consuming large meals close to bedtime can disrupt sleep[21]. Subsequently, poor sleep will affect hormone production and metabolism, potentially influencing vitamin D synthesis and absorption. While the body predominantly synthesizes vitamin D via sun exposure, dietary sources, and supplementation also augment overall levels[22].

Recent studies highlight the importance of assessing food intake, including calorie consumption and energy balance, as crucial components in evaluating vitamin D levels. A positive energy balance, where calorie intake exceeds energy expenditure, can lead to weight gain and an increased BMI. Elevated BMI, often linked to obesity, has been associated with lower circulating vitamin D levels. This is because vitamin D is fat-soluble and thus sequestered in fat tissue, reducing its availability in the bloodstream[23].

Moreover, obesity can impair the body's ability to synthesize vitamin D from sunlight, further exacerbating vitamin D deficiency[24]. A systematic review found that obese individuals had significantly lower vitamin D levels compared to non-obese individuals[25]. Additionally, the sequestration of vitamin D in fat tissue means that individuals with higher BMI have a larger proportion of their vitamin D stored in fat cells, making it less available for metabolic processes[26]. This interconnection suggests that individuals with a positive energy balance and higher BMI may be at greater risk of vitamin D deficiency, further influencing their overall metabolic and reproductive health. As research in this area continues to evolve, it is important to explore together these parameters in addressing the issue of fertility.

The objective of this study is to determine the association between vitamin D deficiency diet intake, chrononutrition profile, and sleep quality among males and females with subfertility.

Materials and methods

A descriptive, cross-sectional, quantitative study was conducted among 140 subfertile individuals at two hospitals in Selangor, Malaysia from March 2023 to March 2024. The sample size was calculated using the standard formula for prevalence studies[27], n = [z^2p (1-p)]/d2 where *z* is the critical value corresponding to a 95% confidence level (1.96), *p* is the estimated prevalence of infertility in Malaysia (10%, based on Vander Borght and Wyns[28], and *d* is the desired margin of error (5%). Substituting these values, the total sample size required was 140.

Participants voluntarily completed a set of validated questionnaires. Inclusion criteria were women diagnosed with infertility under 40 years old and men under 45 years old. Exclusion criteria included individuals with HIV, syphilis, hepatitis B, sexually transmitted diseases (STDs), active thyroid diseases (e.g., Grave's or Hashimoto's), ongoing COVID-19 infection, or active malignancy. The recruitment of this study was conducted by an Obstetrics and Gynaecology (O&G) specialist who attended patients seeking treatment for subfertility. Only patients who met the predefined inclusion and exclusion criteria were selected to participate in the study.

Ethical approval was obtained from the Universiti Teknologi MARA Research Ethics Committee (Reference No. REC/02/2023 (ST/MR/48)). Informed consent was obtained from all participants before their inclusion in the study. They were informed about the study's objectives, the procedures involved, potential risks and benefits, and their right to withdraw at any time without consequences. Consent for the publication of any identifying information was also obtained. The research was conducted in accordance with the Declaration of Helsinki.

Questionnaires

Participants completed bilingual self-administered questionnaires, including a socio-demographic questionnaire, a Chrononutrition Profile Questionnaire (CPQ), a validated Food Frequency Questionnaire (FFQ), and Pittsburgh Sleep Quality Index (PSQI) questionnaire.

The CPQ, originally developed by Veronda et al.[29] was used to assess participants' chrononutrition behaviours by examining how dietary intake aligns with circadian rhythms. For use in the Malaysian population, the questionnaire underwent translation and adaptation to the Malay language and was pilot tested to ensure cultural appropriateness, comprehension, and face validity[30]. This questionnaire evaluates six key dietary patterns: the frequency of skipping breakfast per week, the largest meal that contributes most to daily calorie intake, the timing of the last eating event of the day (evening eating), the duration between the last eating event and sleep onset (evening latency), the frequency of night-time eating, and the eating window, which is the period between the first and last eating events of the day. The behavioral patterns were scored and categorized into three levels: good, fair, and poor, based on their alignment with optimal chrononutrition practices[30].

The FFQ used in this study was adapted from the Malaysian study by Norimah et al.[31], comprising 126 food items categorized into 15 distinct groups. This version accounts for Malaysian dietary patterns and cultural food preferences while assessing dietary intake over the past month. To compute daily food intake, the data was processed using Excel, applying the formula: intake frequency × serving size × weight of food per serving[31]. This method allows for accurate calculation of individual food consumption patterns. Subsequently, the total intake of calories, carbohydrates, proteins, fats, and sugars was analyzed using Nutritionist Pro software, which provides a comprehensive breakdown of the diet's nutritional content.

The PSQI has been translated and validated into many different languages and has been widely used for assessing sleep quality and disturbances over the past month[32]. In this study, we utilized the Malay version of PSQI, which has demonstrated acceptable reliability and validity in Malaysian populations[33]. It consists of 19 self-rated questions that evaluate seven components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction related to sleep. Each component is scored on a scale from 0 to 3, and the total score ranges from 0 to 21. Higher scores indicate poorer sleep quality.

Serum vitamin D assessment

Blood samples (3 mL) were collected from each participant by the staff nurse and further analyzed in the laboratory using an automated immunoassay. The Electrochemiluminescence (ECLIA) method was employed for the quantitative measurement of a total of 25-hydroxyvitamin D in human serum. This assay is used to aid in the assessment of vitamin D status. Serum vitamin D levels were categorized as deficient (< 52 nmol/L), insufficient (52–73 nmol/L), or sufficient (74–250 nmol/L).

Data analysis

Data were analyzed using SPSS software version 29.0, with a significance level set at $p < 0.05$. The distribution of data was checked for normality. Chi-square tests assessed associations between variables, and independent t-tests compared means of continuous variables. Spearman's rho correlation assessed the relationship between dietary intake and serum vitamin D levels, explaining how dietary factors influence vitamin D status. Logistic regression was used to determine the odds ratio for determinants of vitamin D deficiency in a sample of 87 subfertile males and females.

Results

A total of 140 respondents comprising 86 females and 54 males, with a mean age of 33.27 ± 4.12 years, participated in this study. Table 1 presents the demographic data categorized by gender. The demographic distribution revealed that the majority (69.3%) of participants were adults (aged 26–35 years), while 27.9% were middle-aged adults (aged > 36 years) and 2.9% were young adults (aged 18–25 years). Most participants identified as Malay (94.3%), and a significant proportion had attained tertiary education (84.9%). In terms of employment, 79.3% were employed full-time, with 10% being self-employed. Approximately 47.9% had a household income between RM 0 and RM 4,849. Regarding weight changes, 29.3% reported an increase, while 25% reported a decrease in the past month. The Body Mass Index (BMI) average was 27.6 ± 9.7 with 40.7% classified as overweight and 22.1% as obese. Additionally, significant differences were observed between genders ($p < 0.001$), with male has higher percentages of overweight (63.0%) and obesity (18.5%) compared to women.

This study found that a significant portion of respondents had vitamin D deficiency ($n = 87$, 62.1%), while 43 (30.7%) had insufficiency, and only 10 (7.1%) had adequate levels. Table 2 present serum vitamin D status according to gender, with mean serum vitamin D in males averaging 54.19 ± 14.70 nmol/L and females 43.07

Variables	Male ($n = 54$)	Female ($n = 86$)	Total, ($n = 140$)	p-value
Age (years + SD)[a]	34.3 ± 4.5	32.6 ± 3.8	33.3 ± 4.2	0.017*
Age group[b]				
Young adult (18–25)	0	4 (4.7)	4 (2.9)	
Adult (26–35)	35 (64.8)	62 (72.1)	97 (69.3)	0.108
Middle-aged adult (> 36)	19 (35.2)	20 (23.3)	39 (27.9)	
Race[b]				
Malay	49 (90.7)	83 (96.5)	132 (94.3)	
Chinese	1 (1.9)	0	1 (0.7)	0.179
Indian	2 (3.7)	3 (3.5)	5 (3.6)	
Others	2 (3.7)	0	2 (1.4)	
Highest educational level[b]				
Primary	1 (1.9)	2 (2.3)	3 (2.2)	
Secondary	10 (18.9)	8 (9.3)	18 (12.9)	0.263
Tertiary	43 (79.2)	76 (88.4)	119 (84.9)	
Employment status[b]				
Employed full time	45 (83.3)	66 (76.7)	111 (79.3)	
Self-employed	9 (16.7)	5 (5.8)	14 (10.0)	0.001*
Housewife	0	15 (17.4)	15 (10.7)	
Household income[b]				
RM 0 - RM 4849	24 (44.4)	43 (50.0)	67 (47.9)	
RM 4850 - RM 10959	24 (44.4)	33 (38.4)	57 (40.7)	0.770
> RM 10960	6 (11.1)	10 (11.6)	16 (11.4)	
Weight changes in the past month[b]				
Has changes increases	10 (18.5)	31 (36.0)	41 (29.3)	
Has changes decreases	12 (22.2)	23 (26.7)	35 (25.0)	0.026*
No changes	32 (59.3)	32 (37.2)	64 (45.7)	
Body mass index (BMI)[a]	29.2 ± 13.9	26.7 ± 6.1	27.6 ± 9.7	0.148
BMI (kg/m^2)[b]				
Underweight	0	3 (3.5)	3 (2.1)	
Healthy weight	10 (18.5)	39 (45.3)	49 (35.0)	< 0.001*
Overweight	34 (63.0)	23 (26.7)	57 (40.7)	
Obesity	10 (18.5)	21 (4.4)	31 (22.1)	

Table 1. Sociodemographic characteristic. *Significant results are indicated at $p < 0.05$. [a]Data presented in mean ± standard deviation (SD) and analyzed using t t-test to compare means between genders. [b]Data are presented as n (%) and analyzed using a chi-square test to examine the association between sociodemographic characteristics and gender.

	Male	Female	p-value
Mean serum Vitamin D (nmol/L)[a]	54.19 ± 14.70	43.07 ± 16.29	< 0.001*
Serum vitamin D status[b]			
Deficiency (< 52 nmol/L)	21 (38.9)	66 (76.7)	< 0.001*
Insufficiency (52–73 nmol/L)	29 (53.7)	14 (16.3)	
Sufficiency (74–250 nmol/L)	4 (7.4)	6 (7.0)	

Table 2. Serum vitamin D status according to gender. *Significant results are indicated at $p < 0.05$. [a]Data presented in mean ± standard deviation (SD) and analyzed using t t-test to compare means between genders. [b]Data are presented as n (%) and analyzed using a chi-square test to examine the association between sociodemographic characteristics and gender.

± 16.29 nmol/L. A chi-square test indicated a significant relationship between vitamin D categories and gender ($p < 0.001$).

Dietary intake differences between males and females were analyzed using the Mann-Whitney U-Test (Table 3). Males reported a significantly higher median daily energy intake of 1740 kcal compared to 1417 kcal for females ($p = 0.006$). They also consumed significantly more carbohydrates (232.96 g vs. 173.74 g, $p = 0.022$) and fat (63.99 g vs. 43.79 g, $p = 0.002$) than females.

Dietary intake	Male ($n = 54$)		Female ($n = 86$)		*p*-value
	Median	Interquartile range (IQR)	Median	Interquartile range (IQR)	
Energy (kcal)	1740	1380–2805	1417	1082–2030	0.006*
Carbohydrate (g)	232.96	151.41–313.33	173.74	126.28–262.22	0.022*
Carbohydrate (%)	49.62	42.84–53.88	51.66	44.74-56.00	0.237
Sugar (g)	41.73	22.84–73.50	33.48	20.31–75.77	0.469
Sugar (%)	8.88	5.55–13.34	9.62	6.17–14.28	0.199
Protein (g)	91.67	59.66-141.48	77.77	61.57-110.12	0.103
Protein (%)	19.65	17.45–23.34	21.24	17.59–26.56	0.155
Fat (g)	63.99	42.00-89.35	43.79	31.39–67.25	0.002*
Fat (%)	31.00	26.58–33.81	26.45	22.68–30.57	0.010*

Table 3. Dietary intake of the respondents based on gender. *Significant results are indicated at $p < 0.05$. Data was analyzed using the Whitney U Test. IQR = Interquartile range.

	Serum Vitamin D level			
	Male		Female	
	Correlation coefficient, ρ	p-value	Correlation coefficient, ρ	p-value
Energy (kcal)	0.160	0.247	-0.259	0.016*
Total carbohydrate (g)	-0.002	0.986	-0.196	0.070
Total Sugar (g)	0.102	0.465	-0.071	0.516
Total Fat (g)	0.141	0.311	-0.274	0.011*
Total Protein (g)	0.206	0.136	-0.194	0.073

Table 4. Spearman's Rho correlation analysis of dietary intake and vitamin D level. *Significant results are indicated at $p < 0.05$.

Table 4 shows the relationship between dietary intake and serum vitamin D levels using Spearman's rho correlation. In females, there was a significant weak negative correlation between energy intake (ρ = -0.259, p = 0.016) and total fat intake (ρ = -0.274, p = 0.011) with serum vitamin D levels, indicating that higher energy and fat consumption were associated with lower vitamin D levels. Carbohydrates, sugar, and protein intake were not significantly correlated with vitamin D levels in either gender.

Chrononutrition behaviors were compared across different serum vitamin D levels. While most variables showed no significant differences, evening eating habits during workdays (p = 0.001) and eating windows on workdays (p = 0.011) were significantly associated with vitamin D status (Table 5). Participants typically consume their largest meal during lunch.

The relationship between sleep quality and serum vitamin D levels were also explored as shown in Table 6. This study found that no significant differences were found between sleep quality and vitamin D levels (p = 0.108).

Finally, evaluation of the determinants of vitamin D deficiency among 87 subfertile males and females was conducted (Table 7). The analysis shows that females have a significantly higher likelihood of vitamin D deficiency, with an odds ratio (OR) of 5.186 (95% CI: 2.471–10.884, $p < 0.001$), emphasizing the elevated risk in women. Poor evening eating habits during workdays were also strongly associated with vitamin D deficiency, with an OR of 25.778 (95% CI: 2.830–234.815, p = 0.004), and after adjustment, the association remained significant (AOR: 10.553, 95% CI: 1.096–101.649, p = 0.041). In contrast, other factors such as poor sleep quality (AOR: 1.708, p = 0.162), poor eating window during free days (AOR: 0.738, p = 0.607), and BMI ≥ 25.0 kg/m² (AOR: 0.960, p = 0.923) did not show statistically significant associations with vitamin D deficiency after adjustment.

Discussion

Vitamin D is increasingly recognized as a contributing factor to subfertility, affecting both men and women. Studies have shown that vitamin D deficiency can cause hormonal imbalances, ovulatory dysfunction, reduced fertility in women, and impaired sperm quality in men[34,35]. Therefore, maintaining optimal vitamin D levels is essential for both male and female reproductive health.

Globally, the prevalence of vitamin D deficiency has been reported as 24% in the US, 37% in Canada, and 40% in Europe[36]. In Malaysia, the situation is more concerning, with 67.4% of the population affected, including 87% of women and 41% of men in the Malay adult population[37]. Similarly, in this study, 62.1% of respondents were vitamin D deficient, with 38.9% men and 76.7% women.

Vitamin D				
Chrononutrition profile	**Deficiency (n = 87)**	**Insufficiency (n = 43)**	**Sufficient (n = 10)**	***p*-value**
Largest meal				0.576
Breakfast	7 (8.0)	2 (4.7)	2 (20.0)	
Lunch	66 (75.9)	34 (79.1)	7 (70.0)	
Dinner	11 (12.6)	7 (16.3)	1 (10.0)	
Others	3 (3.4)	0 (0.0)	0 (0.0)	
Breakfast skipping				0.543
Good	47 (54.0)	24 (55.8)	3 (30.0)	
Fair	17 (19.5)	8 (18.6)	4 (40.0)	
Poor	23 (26.4)	11 (25.6)	3 (30.0)	
Evening eating (workdays)				0.001*
Good	29 (33.3)	6 (14.0)	3 (30.0)	
Fair	57 (65.5)	29 (67.4)	7 (70.0)	
Poor	1 (1.1)	8 (18.6)	0 (0.0)	
Evening eating (free days)				0.107
Good	23 (26.4)	4 (9.3)	3 (30.0)	
Fair	55 (63.2)	31 (72.1)	7 (70.0)	
Poor	9 (10.3)	8 (18.6)	0 (0.0)	
Evening latency (workdays)				0.127
Good	1 (1.1)	0 (0.0)	0 (0.0)	
Fair	62 (71.3)	22 (51.2)	8 (80.0)	
Poor	24 (27.6)	21 (48.8)	2 (20.0)	
Evening latency (free days)				0.500
Fair	57 (65.5)	26 (60.5)	8 (80.0)	
Poor	30 (34.5)	17 (39.5)	2 (20.0)	
Night-time eating				0.791
Good	79 (90.8)	41 (95.3)	9 (90.0)	
Fair	6 (6.9)	1 (2.3)	1 (10.0)	
Poor	2 (2.3)	1 (2.3)	0 (0.0)	
Eating window (workdays)				0.091
Good	41 (47.1)	29 (20.7)	5 (50.0)	
Fair	35 (40.2)	10 (23.3)	2 (20.0)	
Poor	11 (12.6)	4 (9.3)	3 (30.0)	
Eating window (free days)				0.011*
Good	35 (40.2)	27 (62.8)	1 (10.0)	
Fair	38 (43.7)	11 (25.6)	8 (80.0)	
Poor	14 (16.1)	5 (11.6)	1 (10.0)	
Night-time snacking				0.152
Good	27 (31.0)	18 (41.9)	1 (10.0)	
Fair	32 (36.8)	18 (41.9)	6 (60.0)	
Poor	28 (32.2)	7 (16.3)	3 (30.0)	

Table 5. Association between chrononutrition profile with serum vitamin D status. *Significant results are indicated at $p < 0.05$. Data presented as n (%) and analyzed using a chi-square test to examine the association between chrononutrition profile and vitamin D status. Six main dietary patterns were evaluated: the largest meal that contributes most to daily calorie intake, the frequency of skipping breakfast per week, the timing of the last eating event of the day (evening eating), the duration between the last eating event and sleep onset (evening latency), *the frequency of night-time eating*, and the eating window, which is the period between the first and last eating events of the day.

Vitamin D deficiency is defined by a cutoff level of 50 nmol/L according to the clinical practice guidelines of the Endocrine Society Task Force on Vitamin D[38]. Additionally, various expert bodies and medical societies have agreed this threshold represents the "vitamin D requirement of nearly all normal healthy persons," mainly using bone health as a benchmark[36].

However, to date, there is no universal consensus available on the optimal levels of vitamin D specifically for reproductive health. Studies suggest a generally higher vitamin D levels are required for optimal reproductive health, compared to those required for skeletal health. Research indicates that vitamin D levels of at least 75

Vitamin D				
Sleep quality	Deficiency (n = 87)	Insufficiency (n = 43)	Sufficient (n = 10)	p-value
Good	48 (55.2)	22 (51.2)	2 (20.0)	0.108
Poor	39 (44.8)	21 (48.8)	8 (80.0)	

Table 6. Association between sleep quality and serum vitamin D status. Data are presented as n (%) and analysed using a chi-square test.

Variables	OR (95% CI)	p value	AOR (95% CI)	p value
Female	5.186 (2.471–10.884)	< 0.001*	–	–
Poor sleep quality	1.487 (0.749–2.954)	0.257	1.708 (0.806–3.621)	0.162
Poor evening eating (workdays)	25.778 (2.830–234.815)	0.004*	10.553 (1.096–101.649)	0.041*
Poor eating window (free days)	0.536 (0.182–1.1574)	0.256	0.738 (0.232–2.350)	0.607
BMI ≥ 25.0 kg/m^2	1.530 (0.735–3.183)	0.255	0.960 (0.424–2.175)	0.923

Table 7. Determinants of vitamin D deficiency (n = 87) in subfertile males and females. *Significant results are indicated at $p < 0.05$. AOR = adjusted to gender.

nmol/L are associated with better reproductive outcomes[34]. Another study suggests that vitamin D levels up to 100 nmol/L may be beneficial for reproductive health, especially in reducing complications in pregnant women[16].

In this study, the mean vitamin D levels were 54.19 ± 14.70 nmol/L for men, which categorizes them as having vitamin D insufficiency, and 43.07 ± 16.29 nmol/L for women, which falls into the category of vitamin D deficiency. We also found significant differences in serum vitamin D levels between genders, with males showing higher average levels than females. These findings are in line with previous research that stated differences in males' and females' physiological and behavioral factors, such as outdoor activity and dietary habits[22]. This may explain the observed gender-related differences in vitamin D levels[22].

Vitamin D deficiency is caused by a variety of factors, both environmental and physiological. Inadequate exposure to the sun, which is often due to lifestyle or geographic location, and poor dietary habits are two major contributors. A dietary habit with a lack of vitamin D-rich foods, such as fatty fish, egg yolks, and fortified dairy products, can result in vitamin D deficiency. Additionally, certain medical conditions, such as malabsorption disorders like Crohn's disease and celiac disease, can impair the ability to absorb vitamin D from food[39].

This study further explores the effects of dietary intake on vitamin D levels, specifically by examining energy, total carbohydrate, sugar, fat, and protein consumption. Interestingly, the finding observed gender-specific correlations where a weak but significant negative correlation was found between both energy intake and fat intake in females. This suggests that higher energy and fat intake may potentially interfere with vitamin D levels. The possible explanation for this finding could be due to a dilution effect or the sequestration of vitamin D in adipose tissue, which stores this fat-soluble vitamin[23]. Furthermore, studies indicate that high-fat and high-cholesterol diets can disrupt vitamin D metabolism in the liver by reducing the expression of hepatic vitamin D-25-hydroxylase, an enzyme for converting vitamin D into its active form, 25-hydroxyvitamin D3 (25(OH) D3)[24]. Consequently, it leads to lower serum vitamin D levels. This supports the hypothesis that dietary habits, particularly those that increase body fat, may lower vitamin D levels in females.

Nevertheless, no significant correlations were found between carbohydrates, sugar, or protein intake and vitamin D levels in either gender. This lack of association suggests that, at least in this population, these macronutrients do not have a direct impact on serum vitamin D levels. This is in line with existing literature that points primarily to fat intake and body fat as key factors influencing vitamin D metabolism[40].

This study also examined chrononutrition behaviors as both the types of food consumed, and the timing of meals can influence vitamin D synthesis and absorption. The finding shows that chrononutrition behavior which is evening eating (the timing of the last eating event of the day) during workdays and eating window (the period between the first and last eating events of the day) during free days has a significant impact on serum vitamin D levels.

Individuals with fair evening eating habits were more likely to have vitamin D deficiency and insufficiency compared to those with good habits. These findings suggest that inconsistent or poorly timed evening eating patterns, categorized as "fair," might interfere with optimal vitamin D metabolism or intake, possibly due to late-night meals disrupting circadian rhythms or affecting vitamin D synthesis and absorption[41]. While the poor evening eating habits group had a low rate of vitamin D deficiency, this may be due to the small sample size, and their increased prevalence of insufficiency warrants further investigation.

Similarly, the eating window during free days was significantly associated with serum vitamin D levels. Among participants with a poor eating window, the prevalence of vitamin D deficiency was 16.1%. However, this prevalence was only slightly higher compared to those with vitamin D insufficiency (11.6%) and those with sufficient vitamin D levels (10.0%), indicating minimal differences between the groups. The minimal differences suggest that other factors may also contribute to vitamin D levels in the individuals, which are dietary intake, sunlight exposure, and individual metabolic responses to vitamin D.

There is growing evidence suggesting the bidirectional association between sleep quality and vitamin D levels. Low levels of vitamin D are associated with a higher risk of sleep disorders by disrupting the sleep-wake cycle[42]. This is evidenced by the expression of vitamin D receptors in various brain regions that regulate the sleep-wake cycle, such as the hypothalamus[42,43]. On the other hand, poor sleep habits, such as irregular sleep patterns or insufficient sleep, may have an impact on vitamin D synthesis, because vitamin D synthesis in the skin is regulated by sun exposure, which is lowered in those with poor sleep quality. Despite this theory, this study found no statistically significant association between sleep quality and vitamin D status within this cohort. This suggests that, in this cohort, sleep quality may not have a direct or strong association with vitamin D levels.

Analysis of determinants of vitamin D deficiency in subfertile individuals highlights various risk factors that affect vitamin D levels. Females in this demographic are more than five times as likely as males to be vitamin D deficient. The significant association between female gender and vitamin D deficiency emphasizes the need for gender-specific interventions since females in this cohort are much more vulnerable to low vitamin D levels. This may be attributed to factors such as hormonal differences, lifestyle variations, or dietary habits that warrant further investigation.

Additionally, the strong correlation between poor evening eating habits during workdays and vitamin D deficiency points out the significance of meal timing and dietary content in maintaining optimal vitamin D levels. Conversely, the absence of significant correlations with poor sleep quality, poor eating habits, and BMI shows that these variables may not have a direct impact on vitamin D status in this cohort. Instead, this indicates that factors such as genetics, lifestyle, diet, and choronoutrition, may be more significant in determining vitamin D levels[44].

Chrononutrition behaviors, such as evening eating and eating window may influence vitamin D metabolism by interacting with circadian-regulated processes and affecting nutrient absorption, metabolism and hormone secretion. The circadian rhythm, regulated by the suprachiasmatic nucleus (SCN) in the hypothalamus, orchestrates physiological processes based on light-dark cycles. Late-night eating can misalign the central and peripheral clocks, causing phase shifts that alter melatonin and cortisol secretion, which are critical for sleep-wake regulation and metabolic processes[45]. Melatonin, normally produced at night, is suppressed by late-night food intake, impairing sleep quality and further disrupting the circadian rhythm[46]. This misalignment can dysregulate enzymes involved in converting vitamin D to its active form, calcitriol, reducing the efficiency of vitamin D metabolism. Consequently, chronic circadian disruption and poor sleep quality exacerbate metabolic dysfunction, leading to decreased serum vitamin D levels and associated health implications[47].

Moreover, the length of the eating window plays a role in nutrient absorption. A shorter eating window reduces the time available for vitamin D absorption, whereas a longer window allows more opportunities for nutrient intake and absorption[48]. These findings suggest that vitamin D metabolism may be more intricately tied to circadian rhythm than previously recognized. They also raise questions about whether interventions targeting chrononutrition, such as optimizing meal timing or synchronizing eating patterns with daylight hours could be effective in improving vitamin D status. Overall, these findings lay the foundation for future studies into effective techniques for preventing and treating vitamin D deficiency, particularly in subfertile couples.

However, this study has a limitation, as it does not include known factors that influence vitamin D levels, such as vitamin D intake and sun exposure assessment. These factors are critical since Vitamin D synthesis largely depends on exposure to ultraviolet B (UVB) radiation, which can be significantly influenced by factors such as clothing coverage and the amount of skin exposed[49]. Research indicates that individuals with greater skin coverage due to cultural or religious clothing practices are at a higher risk of vitamin D deficiency[50]. Additionally, inadequate dietary intake of vitamin D-rich foods or supplements can exacerbate low levels, especially in regions with limited dietary fortification[51]. While our study population shares similar environmental conditions and cultural norms influencing clothing choices and sunlight exposure, this generalization might overlook subtle individual variations. The lack of direct assessment of these factors limits our ability to fully interpret the observed variability in vitamin D levels and their potential association with subfertility. Additionally, although the sample was sufficient to detect significant gender differences, the high proportion (90%) of participants with vitamin D deficiency may limit the ability to detect more refined subgroup differences. Future studies should consider factors such as vitamin D intake and sun exposure and use larger, more balanced samples to provide a more thorough understanding of the contributors to vitamin D deficiency.

Conclusions

In conclusion, this study shows the importance of vitamin D in reproductive health, with a significant prevalence of vitamin D deficiency seen among the subfertile population. Subfertile females have a particularly high prevalence of vitamin D deficiency, highlighting the essential of gender-specific interventions. Dietary habits, specifically high energy and fat intake, as well as chrononutrition behaviors such as evening eating patterns, were factors related to vitamin D deficiency. Sleep quality, on the other hand, was not associated with vitamin D levels within this cohort, suggesting that other factors may contribute to a more substantial role. This study expands on the existing knowledge of factors contributing to vitamin D deficiency and underscores the need for a comprehensive approach to identifying and treating vitamin D deficiency, especially in subfertile individuals. Addressing these issues will be vital to enhance reproductive health outcomes and guide public health measures.

Data availability

The datasets generated and analyzed in this study are not publicly available due to participant confidentiality and institutional regulations but are available from the corresponding author on reasonable request and with appropriate institutional approvals.

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Author contributions
S.M.M., N.J., and N.I.M.F.T. conceptualized and designed the study. N.L. and A.N.R. conducted the research, with S.A., J.Y., and A.H.A. provided help and advice on clinical aspects related to subfertility and vitamin D deficiency. Data analysis was carried out by N.L., A.N.R., S.M.M., N.J., and N.I.M.F.T. The manuscript was drafted by S.M.M., N.J., N.I.M.F.T., with all authors contributing to revisions and editorial improvements. All authors have read and approved the final version of the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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