Original Research

Relationship between Dairy Product Consumption and Oligospermia; a Case-Control Study

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Abstract: **Introduction:** Infertility affects an estimated 15% of couples globally and in Iran, a quarter of Introduction: One of the current debates regarding men's reproductive health is the declining sperm count and the role of nutritional and environmental factors. This study aims to investigate the association between dairy product consumption and oligozoospermia, sperm count of 5 to 15 million per milliliter of semen, in adult men.

Materials and Methods: This age-matched case-control study was conducted on 102 oligozoospermia and 306 healthy men (control group). Both groups were recruited from an infertility clinic in Tehran, the Royan Institute. Dietary intake of subjects was collected using a valid and reliable 168-item food frequency questionnaire. Odds ratios (OR), ex-tracted from conditional logistic regression, were used to compare the occurrence of oligozoospermia by tertiles of dairy intake.

Results: After adjusting for potential confounding variables, the high intake of low-fat dairy products was inversely associated with the risk of oligozoospermia (OR: 0.48; 95%CI: 0.24 – 0.95), (P =0.046). The higher intake of high–fat dairy products [(OR: 2.44; 95%CI: 1.26 – 4.73), (P =0.008)], high–fat milk [(OR: 2.16; 95%CI: 1.09 – 4.30), (P =0.043)], and ice creams [(OR: 2.37; 95%CI: 1.25 – 4.50), (P =0.008)] were also positively associated with oligozo-ospermia.

Conclusion: The high intake of low–fat dairy foods seems to have a protective effect on oligozoospermia. Higher intake of high – fat dairy products, high– fat milk, and ice creams were associated with an increased risk of oligozo-ospermia.

Keyword: Oligozoospermia; low sperm count; dairy; low fat dairy

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1. Introduction

Couples unable to conceive after 12 months of unprotected intercourse are referred to as "infertile" [1]. Infertility is a social and medical issue all around the globe which negatively affects couples' psychological and medical status.

The prevalence of infertility has been estimated at 8-15.8% in the **world** and 14-20% in Iran [1-3].

According to the current literatures, 50% of all infertil-

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ity causes are related to male factors [4]. To study infertility in men, semen's quality is examined, during which the count, density, motility and morphology of sperms are investigated. One of the current discussions regarding fertility health in men is the descending trend of sperm count [5], which is known as "Oligospermia" and is defined as 5-15 million sperms per millimeter of semen (average and mild oligospermia)[6].

There have been multiple factors suggested by the researchers that cause this issue, of which low physical activity, high sedentary activity [7], obesity [8] and dietary intake [9] are the most significant.

Considering the importance of regular consumption of dairy products in the diet, an increasing trend has been observed in dairy intake and particularly low-fat dairy products [10]. While some researchers have reported a positive association between increased dairy product consumption and sperm count and density, others have observed a negative association or even failed to detect any relation between the two [11][12].

These variations could be attributed to the differences between the target populations of these studies; whether they had recruited healthy or sub fertile individuals, the sample size and the settings of the studies, as some surveys have only evaluated the number of times their participants have consumed dietary products without including a supplementary analysis to investigate the exact association [11-13]. Considering the scarcity of available literature about this issue, and particularly shortage of evidence on Iranian population we aimed to conduct the present study.

2. Method

This age-matched case-control study was conducted on a sample of male individuals aged 18-45 years old, referring to Tehran's Royan Institute. A total of 408 eligible men (102 cases and 306 controls) were invited to participate in the study. Male subjects with clinically-proven oligospermia (sperm count of 5-15 million per millimeter of semen fluid [6]), with no particular diet who were willing to participate in the study comprised the case group of this survey. For each oligospermic case, 3 healthy men (the control group) were recruited as well. The men in the control group had a sperm count of 15 million or more per millimeter, total sperm motility (A+B) of more than 32% and normal morphology of more than 4%. All the semen samples were collected in the same laboratory and were examined under standard conditions [14, 15]. All the participants of this research were only included in the study after becoming fully aware of the aims and methods of the research and signing an informed written consent.

As mentioned, 448 men were initially invited to participate, of which 40 either disagreed initially or decided to withdraw after filling 1 or 2 pages of the food frequency questionnaire (FFQ), rendering a participation rate of 91%.

Demographic characteristics, dietary intake and physical activity data were gathered from all the participants. Dietary intake of the subjects for the past year was gathered via a valid and reliable 168-item FFQ by an experienced and trained interviewer. In the frequency and dietary intake interviews, the participants were asked to report the daily, weekly, monthly or yearly consumption frequency of each food item for the past year. The reported consumption frequency of each food item was converted in grams of the ingredients. After completion and conversion of data to grams per day, each food item was converted to a unit via USDA [16], and consumption serving of each subgroup was measured. Later on, final dairy consumption quantity of each participant was calculated through summation of all units. Low-fat dairy products included Doogh (yogurt drink) and low-fat types of cheese, milk and yogurt and high-fat dairy products included yogurt and high-fat cheese, cream yogurt and different types of ice-creams.

Anthropometric measurements like height and weight of the individuals were measured based on standard protocols, using a digital-Seca scale with 100g accuracy. The height of the participants was also measured with the stadiometer installed on the weight scale with an accuracy of 0.1 cm, with the subjects' standing straight, their shoulders at their normal position, and shoes removed. The body mass index (BMI) of each individual was calculated by dividing weight (kg) by height (m) squared. The waist circumference was also measured between the lowest rib and iliac crest with an inelastic tape measure. Daily physical activities were examined via International Physical Activity Questionnaire (IPAQ) via face-to-face interviews.

At the end, metabolic equivalent of task (MET) for each activity was multiplied in time duration of the task (minute) and then with summation of all the calculations, the average activity was estimated for each participant's previous week. The figures were then divided by 60, which resulted in the metabolic equivalent of task in hour.

The participants who did not answer more than 40% of the food frequency questionnaire (15 persons) and those with an energy to the basal metabolic rate ratio of less than 1.2 (17 persons; 7 cases and 19 controls) or more than 2.4 (15 persons; 3 case and 12 control) were excluded from the study (20-21). In the final analysis, 376 subjects (93 cases and 283 controls) were included.

The statistical analysis was done via SPSS software version 20. To determine the significance of difference in qualitative variables between the two groups, Chi-

square test was used. Quantitative data with normal distribution were presented as mean ± Standard deviation and their differences between the two groups were examined by T-test, while the data with non-normal distribution were expressed as Median ± Interquartile range and compared between the groups via Mann Whitney-U test.

To estimate the association between dairy consumption and oligospermia, conditional logistic regression was used and its findings were reported as odd ratios with 95% confidence interval.

3. Result

In this matched case-control study, average age, BMI and energy intake in the case and control groups were, 33.3 vs. 33 years, 26.6 vs. 26.6 kg/m2 and 3220 vs. 3230 kcal/day, respectively. The majority of the participants (approximately 78%) had high school or university education. Physical activity was higher among the case group compared to controls (36 vs. 26 Met h/week, p=0/64), but this difference was not statistically significant (table 1).

In order to estimate the association between tertiles of intake for all dairy products (including all dairy consumption of individuals containing low and high fat milk, yogurt and cheese) and each subgroup of total dairy intake with oligospermia, conditional logistic regression analysis was performed, with and without confounding variables of age, BMI, physical activity, duration since last ejaculation and energy intake. (Table 2)

The variable of age was also added to the adjusted model, even though it had been matched. There were primarily two reasons to add age to the models; first, during the matching, age groups were used instead of the actual age. Secondly, sperm count lowers by a yearly basis as individuals get older. To control this effect, age was adjusted.

The odds of having oligospermia in subjects of the third tertile of low-fat dairy intake was significantly lower than the subjects of the first tertile (OR=0.54, 95% CI: 0.30-0.99, p=0.046), the odds ratio of which further de creased after controlling for confounding variables (OR=0.48, 95% CI: 0.24-0.95, p=0.037)

Higher consumption of high-fat dairies was positively associated with higher odds of oligospermia in the participants. Subjects of the third tertile of high-fat dairy consumption were found to have nearly twice the odds of having oligospermia compared to the subjects of the first tertile (OR=2.07, 95% CI: 1.15-3.75, p=0.015).

This association appeared stronger after adjusting for the confounding variables. (OR=2.44, 95% CI: 1.4-4.73, p=0.008).

Higher consumption of ice-cream was also in direct relation to oligospermia, as the odds of being oligospermic among people in the third tertile of ice-cream consumption was 2.50 times that of the subjects in the first tertile (OR=2.50, 95% CI:1.36-4.46, p=0.002), which decreased to 2.37 after adjustments (OR=2.37, 95% CI:1.25-4.50, p=0.008)

Higher consumption of high-fat milk and dairy products were also positively associated with increased rate of oligospermia. The odds of having oligospermia in the subjects of the third tertile of high-fat milk consumption was 1.8 times that of the participants in the first tertile (OR=1.80, 95% CI:0.98-3.30, p=0.084), which increased to 2.16 times after controlling for confounding variables (OR=2.16, 95% CI:1.26-4.73, p=0.043).

No association was found between higher total consumption of dairy, low-fat milk, and different types of yogurt and cheese with oligospermia.

4. Discussion

In this age-matched case-control study, we investigated the relationship between dairy products' consumption

Table 1: Demographic and anthropometric characteristics of Oligospermia and control groups.

	Control (n=283)	Oligospermia (n=93)	*P value
Age (y) **	33. ± 4.9	33.2 ± 4.6	0.25
BMI (kg/m2) **	26.6 ± 3.7	26.6 ± 3.8	0.98
Education, n (%)***			0.80
Primary school	59 (20.9)	21 (22.6)	
High school	115 (40.8)	28 (30.1)	
Higher education	108 (38.3)	44 (47.3)	
Physical activity (MET-h/day) **	26 (17-71)	36 (18-84)	0.64
Energy intake (kcal/day) **	3230 (771)	3220 (675)	0.90
Occupation, n (%)***			0.17
Employee	120 (42.6)	41 (44.1)	
Others	162 (57.4)	52 (55.9)	
Smoking, n (%)***			0.49
Yes	64 (22.7)	18 (19.4)	
No	219 (77.3)	75 (80.6)	

* Chi-squared test for categorically distributed variables and Student's t test for variables with normal distributions, ** mean ± standard deviation, *** number (percent); BMI; body mass index, MET-h/day; Metabolic Equivalent / hour per week.

Table 1: Odds Ratio of Oligospermia and Confidence Interval of 95% based on dairy intake (Serving for 1000 Kcal per day).

	Tertiles of dairy intake			
	T1	T2	T3	P value
Dairy group				
Model 1	1.00	1.12 (0.63-2.00)	1.01 (0.57-1.07)	0.70
Model 2	1.00	1.69 (0.62-2.20)	1.25 (0.65-2.04)	0.80
Low fat dairy				
Model 1	1.00	0.90 (0.50-1.60)	0.54 (0.30-0.99)	0.046
Model 2	1.00	0.88 (0.48-1.63)	0.48 (0.24-0.95)	0.037
High fat dairy				
Model 1	1.00	1.56 (0.87-2.90)	2.07 (1.15-3.75)	0.015
Model 2	1.00	1.71 (0.85-3.42)	2.44 (1.26-4.73)	0.008
Low fat milk				
Model 1	1.00	0.63 (0.34-1.15)	0.77 (0.37-1.60)	0.410
Model 2	1.00	0.70 (0.37-1.38)	0.84 (0.40-1.78)	0.440
High fat milk				
Model 1	1.00	2.24 (1.22-4.02)	1.80 (0.98-3.30)	0.84
Model 2	1.00	2.27 (1.37-5.40)	2.16 (1.26-4.73)	0.043
Low fat yogurt				
Model 1	1.00	0.70 (0.40-1.23)	0.75 (0.43-1.31)	0.30
Model 2	1.00	0.62 (0.33-1.17)	0.70 (0.40-1.30)	0.24
High fat yogurt				
Model 1	1.00	0.87 (0.48-1.59)	1.28 (0.72-2.26)	0.40
Model 2	1.00	0.87 (0.45-1.71)	1.30 (0.68-2.50)	0.38
Ice cream				
Model 1	1.00	1.30 (0.70-2.24)	2.50 (1.36-4.46)	0.002
Model 2	1.00	1.35 (0.68-2.64)	2.37 (1.25-4.50)	0.008
Total cheese				
Model 1	1.00	0.74 (0.41-1.32)	0.80 (0.45-1.44)	0.31
Model 2	1.00	1.19 (0.41-2.30)	1.36 (0.72-2.60)	0.31
Low fat cheese				
Model 1	1.00	1.36 (0.77-2.40)	1.07 (0.60-1.93)	0.77
Model 2	1.00	1.33 (0.72-2.46)	0.97 (0.52-1.83)	0.95
High fat cheese				
Model 1	1.00	1.87 (0.76-2.50)	1.09 (0.72-2.26)	0.67
Model 2	1.00	1.13 (0.60-2.13)	1.12 (0.68-2.50)	0.64

Data are odds ratio and 95% confidence interval; Logistic regression models were used: Model 1: crude model; Model 2: Additional adjustment for energy intake (kcal/d), physical activity (Met-h/day), BMI (kg/m2), age (y) and abstinence time.

with oligospermia. Based on our findings, the risk lowered with increases in the total low-fat dairy consumption tertiles, while no significant difference was found with individual increases in consumption of low-fat milk, yogurt and low-fat cheese. With increases in high-fat dairy intake, high-fat milk and ice-cream, the risk of oligospermia increased; although intake of different yogurts, cheeses and all dairy products were not associated with presence of oligospermia. These associations remained statistically significant after adjusting for confounding factors such as age, BMI, energy intake, time since the last ejaculation and physical activity. The findings of our research relatively confirm the reports of previous studies regarding the association between low-fat dairy intake and subfertility in men. In our findings, higher consumption of low-fat dairy was negatively associated with the risk of oligospermia, which is congruent with the findings of Afeiche et al.'s study [13], in which they evaluated sub fertile men and reported that a higher consumption of low-fat dairy products is in a positive association with density and progressive motility of sperms, in a way that men in the highest quartile of total dairy consumption (1.22-3.54 serving per day) had 33% more sperm density compared to the subjects of the first quartile. In our research, low-fat milk consumption had an inverse association with oligospermia which was not statistically significant and was contradictory to the results of Mendiola et al.'s research [17], who reported higher low-fat milk consumption in oligoasthenoterato-spermic men compared to healthy men. Afeiche et al. [13] found a positive association between increases in low-fat milk consumption and total sperm count, density and progressive motility of sperms. Median and interquartile range of low-fat milk in our research was estimated at 0.41 (0.27-0.94) servings per day which is much less than the figures of 0.80-2.64 servings per day (highest low-fat milk quartile) reported in Afeiche et al.'s research [13], which could be a factor in our failure to achieve those results.

We found a strong positive association between increases in total high-fat dairy consumption and oligospermia. Afeiche et al. [11]too, observed a negative but insignificant relation between total high-fat dairy consumption in intake quartile (4th quartile with 0.57-5.45 servings per day) and all the semen quality factors.
In another cross sectional study, Afeiche et al. evaluated 189 university students aged between 18 to 22 years old and they found a negative but marginal relation between increases in consumption of high-fat dairies with sperm count and density.
Considering the findings of the two mentioned studies (in the first research insignificant and in the second one marginally significant) and due to their cross-sectional setting and sample size limitations, we attempted to conduct a more comprehensive research. In our study, with increases in ice-cream consumption, the odds of oligospermia also increased. The subjects in the third tertile had 2.37 times odds of oligospermia compared to the subjects of the first tertile. Only Afeiche et al. investigated the relation between ice-cream consumption and semen quality, which had no relation to any of the contributing factors of the semen quality. In our study, we found no association between diary product consumption and oligospermia, which is compatible with the findings of Vujkovic et al.'s cross-sectional study [12], in which 161 low fertile men were evaluated, and no association was found between total diary product consumption and any of the semen quality factors. On the other hand, Afeiche et al. found a negative and marginal relation between total dairy consumption and count and density of sperms. Mendiola et al. [17] showed a higher total dairy consumption (whole milk, semi skimmed milk, cheese and yogurt) in people with oligoasthenoteratospermia compared to healthy people. Although that research included a limited sample population and did not involve analyses to compare the two groups, their results were incongruent with ours.
One of the possible reasons we were not able to detect a relation between sum of total low-fat and high-fat dairy consumption might lie in the inverse association of these two, individually, with oligospermia, which is not related to oligospermia when considered as total dairy consumption. In our research, we found no relation between yogurt consumption and oligospermia, which is compatible with the findings of Afeiche et al.'s study conducted in 2012 [11] and is not congruent with that of Mendiola et al.'s [17] and Afeiche et al.'s research in 2014 [13].
There have been certain mechanisms suggested in regards to the higher odds of oligospermia in subjects consuming high-fat dairy products and the possible protective effect of low-fat dairies against oligospermia. Chemicals like Dioxin, Polychlorinated Biphenyl, and Polyflourenes accumulate in fats and are known to disrupt the Endocrine system and have the ability to influence semen's quality [18-21].
Commercial milks are combination of female cows' milk in different stages of pregnancy which naturally contains Estrogen with Placental sources. The received Estrogen causes negative feedback on LH and FSH, which then results in change in sperm production. However, in some studies, higher intake of dairy products has been associated with increases in FSH serum levels, which can negatively affect testicles and eventually lead to decreased sperm production [11].
The Amino acidic compounds, bioactive chemicals and calcium contents of dairy products can affect Insulin and IGF-1 secretion that need to attach to insulin receptors in Leydig cells and activate them in order for the spermatogenesis to process, which can possibly explain the observed relation between low-fat milk and higher sperm density in humans [13, 22, 23].
The strength of this research lies in using valid and reliable questionnaire to gather data on the dietary intake of participants during the past year, and the case-control setting of the study design which enabled us to better compare associations. Another factor in the strength of this research was controlling the observed relations for confounding factors that can affect sperm quality such as age, body mass index, abstinence time and physical activity of individuals. Also, sampling from one of the referral infertility centers in Iran, provided us with a sample population that could be considered representative of the sub fertile Iranian men all around the country.
Of the limitations of this study was application of the food frequency questionnaire which can introduce recall bias to the results of the survey. Although the sample population were recruited from a referral infertility center, but still the participants who refer to the infertility center do not represent all men in the society and the data obtained from them cannot be generalized.

5. Conclusion:

To conclude, this research showed that increasing consumption of low-fat dairies is associated with decreases in the risk of oligospermia and increasing consumption of high-fat dairies, high-fat milk, and ice-cream, are associated with increases in the risk of oligospermia. It is recommended that in order to confirm or reject the relations found in this research, more extensive and comprehensive studies be conducted.

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7. Conflict of interest:

All authors declare that there is no conflict of interest in this study.

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9. Author's contributions:

Project development, Alireza Dizavi1, Hassan Eini-Zinab, Jalil Hosseini; Data collection and data analysis, Nasrin Omidvar and Alireza Dizavi; Manuscript writing, Jalil Hosseini; Manuscript editing, Alireza Dizavi1, Hassan Eini-Zinab, Jalil Hosseini, Nasrin Omidvar and Moham-mad Ali Hosseini.

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