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# Food intake diet and sperm characteristics in a blue zone: a Loma Linda Study☆

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ABSTRACT

*Objectives:* The study examined the effect the life-long vegetarian diet on male fertility and focused on vegetarians living in the Loma Linda blue zone, a demographic area known for life longevity. The objective was to compare sperm characteristics of vegetarian with non-vegetarian males.
*Study design:* The cross-sectional observational study was based on semen analyses of 474 males from 2009 to 2013. Patients categorized themselves as either life-long lacto-ovo vegetarians ($N = 26$; vegetable diet with dairy and egg products), vegans ($N = 5$; strictly vegetables with no animal products) or non-vegetarians ($N = 443$; no diet restrictions). Sperm quality was assessed using a computer-aided sperm analyzer and strict morphology and chromatin integrity were manually evaluated.
*Results:* Lacto-ovo vegetarians had lower sperm concentration (50.7 ± 7.4 M/mL versus non-vegetarians 69.6 ± 3.2 M/mL, mean ± S.E.M.). Total motility was lower in the lacto-ovo and vegan groups (33.2 ± 3.8% and 51.8 ± 13.4% respectively) versus non-vegetarians (58.2 ± 1.0%). Vegans had lowest hyperactive motility (0.8 ± 0.7% versus lacto-ovo 5.2 ± 1.2 and non-vegetarians 4.8 ± 0.3%). Sperm strict morphologies were similar for the 3 groups. There were no differences in rapid progression and chromatin integrity.
*Conclusions:* The study showed that the vegetables-based food intake decreased sperm quality. In particular, a reduction in sperm quality in male factor patients would be clinically significant and would require review. Furthermore, inadequate sperm hyperactivation in vegans suggested compromised membrane calcium selective channels. However, the study results are cautiously interpreted and more corroborative studies are needed.



## Introduction

Improper diet and defective nutrition has been linked to over 60 diseases in humans, including cancer, obesity, diabetes and inflammatory responses [1–3]. There has been considerable interest in the impact of a strict vegetarian diet on improving health. Individuals on a vegetarian diet or vegetarians, practice abstinence from the consumption of meat – red meat, poultry, seafood and all other animal flesh – for a variety of reasons – cultural, environmental, economic, health-related, political or religious. Variations to the vegetarian diet exist such as ovo-vegetarians that consume eggs but not dairy products, and lacto-ovo vegetarians that include both dairy and eggs in their diet [4]. In contrast, vegans are very strict vegetarians that consume plant products only and exclude meat, eggs, dairy and animal-derived ingredients in their diet. Commonly, all vegetarians substitute soy into their diet for meat products to maintain protein consumption.

A vegetarian diet rich in soy foods has been recognized for decades to provide health benefits, most recently to have favorable effects on metabolic parameters and cardiovascular risks, but its role in male fertility remains controversial [5]. The concern lies in the fact that isoflavones in soy foods exert estrogen-like effects on sperm in vitro and in-vivo bringing about the possible adverse effects of infertility and feminization in men who consume soy products [6,7]. A study reported in 2008 concerning semen quality in men from an infertility clinic in Boston showed an inverse association between soy food intake and sperm concentration and brought more attention to this issue [8].

Unfortunately, most of the studies of fertility and the effects of an increased soy-containing diet do not extend past 3 months of observation, and the amount of soy-intake varies significantly from

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one study to another. One must however consider the possibility that long term exposure to isoflavones and phytoestrogens would have a more significant impact on male reproductive cells. In particular, life-long exposure to estrogen-like compounds would have a more potent effect during puberty. It is this idea that fosters male factor studies in men who have been vegetarians for the majority of their lives and who are also having issues with infertility.

The population at the infertility center where this study was conducted included Seventh Day Adventists (SDA) whose religion supported life-long vegetarianism. This location has been designated a Blue Zones National Geographic demographic region where people have long life spans [9]. This is an important point to study the possible link between diet and human life span. Californian Seventh Day Adventist vegetarians eat an average of 3.5 servings of meat substitutes, which usually contain soy per week [10]. The objective of this case-control observational study was to investigate the effect of the vegetarian diet on sperm characteristics in male patients attending a fertility clinic. The information from this study would assist clinicians in counseling patients on different choices of healthy diets promoting male fertility.

**Table 1**

Semen analysis parameters in 474 male patients identifying as non-vegetarians, lacto-ovo vegetarians and vegans in a blue zone fertility clinic. Values are expressed as mean ± S.E.M.

| Parameter | Non-vegetarians | Lacto-ovo vegetarians | Vegans |
|---|---|---|---|
| No. of specimens (*N*) | 443 | 26 | 5 |
| Male age (years) | 35.7 ± 0.3 | 36.2 ± 1.1 | 40.8 ± 6.9 |
| Abstinence (days) | 4.0 ± 0.1 | 4.9 ± 1.1 | 3.8 ± 1.6 |
| Volume (mL) | 3.1 ± 0.2 | 3.4 ± 0.3 | 4.1 ± 0.8 |
| Concentration (Million/mL) | 69.6 ± 3.2 | 50.7 ± 7.4* | 51.0 ± 13.1 |
| Total motility (%) | 58.2 ± 1.0 | 33.2 ± 3.8* | 51.8 ± 13.4 |
| Rapid progression (%) | 34.7 ± 1.0 | 36.4 ± 3.5 | 30.3 ± 11.1 |
| Curvilinear velocity (μm/s) | 43.1 ± 0.5 | 43.6 ± 2.1 | 41.0 ± 2.6 |
| Linearity (%) | 33.3 ± 0.4 | 33.6 ± 1.4 | 30.4 ± 2.1 |
| Strict normal morphology (%) | 8.1 ± 0.3 | 7.4 ± 1.0 | 6.7 ± 2.4 |
| Area of sperm head ($\mu m^2$) | 10.8 ± 0.1 | 11.2 ± 0.5 | 11.2 ± 1.3 |
| Hyperactivation (%) | 4.8 ± 0.3 | 5.2 ± 1.2 | 0.8 ± 0.7* |
| Normal chromatin integrity (%) | 73.1 ± 0.6 | 71.2 ± 4.1 | 78.7 ± 2.3 |

* $p < 0.05$. Different from non-vegetarians.

## Materials and methods

### *Semen collection and analyses*

Routine diagnostic semen analyses based on W.H.O. 4th Edition guidelines [11] were carried out in the male partner of 474 couples attending a fertility clinic during a 5-year period from 2009 to 2013. All couples had documented history of infertility issues. For consistency, the same laboratory technician performed the semen analyses during that time period. This cross-sectional observational study was approved by the Loma Linda University Institutional Review Board and consents were previously obtained from the patients. In terms of daily diet, patients categorized themselves as either lacto-ovo vegetarians, vegans, or non-vegetarians. The lacto-ovo vegetarian group included pesco vegetarian. Lacto-ovo vegetarians ($N = 26$) were males on a vegetable diet that included soy, nuts, beans, dairy and egg products. Vegans ($N = 5$) were males on strict vegetable diet that included soy, nuts, and no animal food products. Non-vegetarians ($N = 443$) were males with no diet restrictions. At this fertility clinic, the lacto-ovo vegetarian males were 81% non-Hispanic white, 15% South Asia Indian and 4% Hispanic while the 5 vegan males were 100% non-Hispanic white. The non-vegetarian males were 53% non-Hispanic white, 23% Black/African-American, 18% Hispanic, 1% South Asia Indian and 5% Asians. The mean abstinence period was similar for all groups ($p > 0.05$, Table 1).

Exclusion criteria included specimens from males that did not provide vegetarian status information, specimens from micro-epididymal sperm aspiration procedures, testicular extraction and donor specimens. The healthy lifestyle at the Loma Linda region was associated with minimalized confounding factor effects such as smoking (1.1%), mean exercise levels of 50% higher than the U.S. national average [12] and healthy body mass index (BMI) and physical activity [4,10,13].

The mean concentration of sperm cells for each patient was determined from several random microscopic fields in a Brightline hemocytometer counting chamber (Hausser Scientific, Horsham, PA). The sperm motility parameters were assessed in the unwashed sperm cells within an hour after collection using the Hamilton Thorn HTM-C (Hamilton-Thorn Corporation, Danvers, MA) computer-aided sperm analyzer (CASA) as previously reported [14]. Briefly, each semen specimen was mixed, a drop aliquots (10 μL) placed on a glass slide pre-warmed at 37 °C and covered with a cover slip and examined using phase contrast (250×) microscope with CASA attachments [14]. Sperm parameters associated with fertility were determined: percent rapid progression, total motility, linearity, curvilinear velocity and the mean area of the sperm head.

Sperm strict normal morphology was manually assessed. Basically, unwashed smears of sperm were made on glass slides, air-dried and processed using the modified Wright-Giemsa protocol (Diff-Quik Stain Set, Siemens Healthcare Diagnostics Inc., Newark, DE) as previously reported [15,16]. The strict criteria consisted of classifying a sperm as normal when the head (width: 4–6 μm; length 5–10 μm) was oval with the acrosome occupying 40–70% of the head, absence of midpiece and tail defects and absent or negligible cytoplasmic droplets. At least 100 sperm cells were assessed for percent strict normal morphology according to the Tygerberg strict criteria developed by Kruger and colleagues [15]. Stained sperm were analyzed under oil immersion at 1000× objective power microscopy and data analyzed as described below.

### *Hyperactivation analysis*

An aliquot (10 μL) of each liquefied semen was placed into a micro-centrifuge tube containing the 0.5 mL of modified human tubal fluid (HEPES - HTF) medium with 5% serum substitute supplement (Irvine Scientific, Santa Ana, CA) and incubated at 40 °C for 20 min. After incubation, an aliquot (10 uL) was removed from the topmost layer of the incubated mixture, placed on a pre-warmed glass slide and a cover slip was placed over the droplet. Hyperactivation [17] characterized by star-spin or high amplitude of lateral head displacement motility was measured using the sort module on the HTM-C analyzer. Hyperactivation motility is a marker of functional CatSper calcium selective channels on the sperm membrane and precedes capacitation [18]. The result of each specimen was expressed as percent hyperactive sperm in the population of motile sperm.

### *Analysis of sperm chromatin abnormalities*

The staining protocol was based on the published Diff-Quik protocol for the evaluation of sperm morphology and chromatin status [19]. Stained sperm were analyzed under oil immersion at 1000× objective power microscopy. The percentage of sperm with intact chromatin was determined from the ratio of the number of sperm with normally-stained light purple nuclei (type A) to the total number of sperm analyzed.

*Statistical analyses*

Statistical analyses and sample size requirement were performed using OpenEpi, an online statistical program (www.openepi.com). Power for the sample size tested was determined to be 74.7%. The Kolmogorov-Smirnov test of normality was used to test data distributions. ANOVA was carried out for groups followed by student's two-tailed *t*-test statistics. Differences with values of $p < 0.05$ were considered statistically significant. The results in Table 1 were expressed as mean ± S.E.M.

## Results

Over the course of the study period, specimens were collected from 26 self-reported lacto-ovo vegetarians, 5 vegans and 443 non-vegetarians (Table 1). The participation response rate was 51.9% for the 5-year study period. Mean male age and days of abstinence of the subjects at the time of specimen collection were similar across the three groups.

Lacto-ovo vegetarians had significantly lower sperm concentration (50.7 ± 7.4 million/mL, mean ± S.E.M.) when compared with non-vegetarians (69.6 ± 3.2 million/mL). Furthermore, total motility was lower in the lacto-ovo group (33.2 ± 3.8% versus non-vegetarian 58.2 ± 1.0%). Although vegans had numerically lower total sperm motility (51.8 ± 13.4%) and lower concentration (51.0 ± 13.1 million/mL) the results were not significantly different. Interestingly, hyperactive motility was lowest in the vegan group (0.8 ± 0.7%). However, among the 3 groups, the vegan group had the highest percent normal chromatin integrity (78.7 ± 2.3). The percent strict normal sperm morphology was similar in all groups and were within the W.H.O. normal reference range. There were no differences in the remaining sperm parameters (Table 1).

## Comment

The results demonstrated lower sperm concentration and total motility in lacto-ovo vegetarians when compared with non-vegetarians. The vegan group also showed numerically lower sperm concentration and motility. The results from the vegan group was included as there is a paucity of data for male factor in vegans, for example, a search in PubMed.gov revealed only 6 studies of vegan sperm in its entire database. In the vegan group, hyperactive motility, a marker of sperm activation occurring prior to the oocyte fertilization event, was significantly lower when compared with the other groups. This suggested that the CatSper voltage-sensitive calcium selective channels [18] responsible for hyperactivation motility were compromised in the sperm of vegans. The significance of this finding remains unknown but points toward deficiencies in calcium transport and associated diseases which have been extensively reviewed elsewhere [20]. The results for vegans need to be carefully interpreted in light of the small number of vegans studied.

Lacto-ovo vegetarians consume more soy products than non-vegetarians for protein substitution. It is postulated that phytoestrogens and isoflavones in soy may be exerting a negative effect on sperm quality. Supporting evidence can be found in the Harvard School of Public Health 2008 study of semen quality in men from an infertility clinic which showed an inverse association between soy food intake and sperm concentration [8]. Data from a study in India of infertile men showed higher xenoestrogen concentrations in fish eaters and vegetarians [21]. Further suspicions of soy influences are raised by animal studies, one of which showed that infant marmoset monkeys fed on soy formula had decreased serum testosterone concentrations during adulthood influencing their fertility [22]. Another study in male rats showed disruption of steroid regulation of the epididymis by phytoestrogens, which demonstrated reduced fecundity [23]. It may therefore be important to discuss decreasing soy product intake with male partners of infertile couples, particularly those diagnosed with male factor infertility.

In contrast, case reports also exist supporting phytoestrogens for promoting fertility in men [24]. Additional studies in which men were randomized to receive low or high doses of isoflavone supplementation to their diets found no significant differences in sperm parameters between the two groups [25,26]. Chavarro and colleagues [8] showed that soy had no effect on sperm morphology or motility, both of which are believed to impact male fertility. The study, however, only looked at the intake of soy-based foods by male partners of subfertile couples over a 3 month period. One must consider the possibility that exposure to isoflavones should be more prolonged to observe significant effects on sperm. For instance, S-equol, a product of human intestinal bacterial synthesis from soy isoflavones [27], has a high affinity for estrogen receptor β, which is present in human testicular cells and ejaculated human sperm [28,29]. Clearly, a longer duration of soy intake is needed to affect the many sequential steps of spermatogenesis with each step involving accurate gene expression to produce a single normal sperm. Altogether, the time to make a single sperm is about 74 days and sufficient numbers of sperm must be produced to show any changes in the sperm parameters. Accordingly, this study showed sperm motility to be significantly lower in lacto-ovo vegetarians. This may be due to the SDA population of patients whose religion supports life-long vegetarianism and confers possible effects of long term phytoestrogen exposure on sperm quality.

It is speculated that the results observed in vegetarians may be related to pesticide exposure. Fraser's study [10] of SDA diet types and health benefits showed that vegetarian SDA patients consumed more tomatoes, legumes, nuts and fruit than their SDA non-vegetarian counterparts. Supporting evidence comes from a recent study of fruits and vegetable pesticide residue amounts and lower total sperm count and lower percentage of morphologically normal sperm [29]. Even though vegetarians are consuming more fruits and vegetables, they are not necessarily consuming organic produce which has lower reported pesticide residues than conventionally grown foods [30,31]. A study from Denmark found that men who consumed conventionally grown fruits and vegetables had a lower portion of morphologically normal spermatozoa than men who consumed organic diets [32]. In this study, the percentages of sperm with normal morphology were numerically lower in both the lacto-ovo vegetarians and vegans groups when compared with the non-vegetarians group but they did not reach statistical significance.

The evidence thus so far supported the presence of estrogenic compounds or chemical residues in the vegetarian diet as the main cause of reduced sperm parameters, namely, concentration and motility. However, the absence or reduced intake of dietary nutrients such as vitamin B12 and zinc by vegetarians may also be another possible explanation for the reduced sperm parameters [33]. These dietary nutrients which are lacking in vegetarians have been shown to be essential for sperm production and function. More studies are necessary to elucidate the precise cause of reduced sperm parameters in the vegetarians.

The strength of this study included the unique data from a specific population of males in a blue zone region. However, a limitation of studying life-long vegetarians was the inherent difficult process of controlling the randomization of diets in males that start at birth. Other confounding factors such as body mass index (BMI), physical activity or smoking, have already been reported for this population [4,10,13,34].

In summary, the vegetarian diet decreased sperm quality in males. More importantly however, for male factor patients in the groups studied here, a reduction in sperm quality would be clinically significant and would require review and patient counseling. Furthermore, inadequate sperm hyperactivation in vegans suggested compromised membrane calcium selective channel which would require prompt attention and alteration in dietary supplementation. Evidence-based lifestyle adjustments which positively influence a patient's ability to conceive are excellent tools but recommendations for diet modification require further studies and confirmation of current findings.

## Conflict of interest

All the authors declare they have no financial and personal relationships with other people or organizations that could influence this research work.

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