Original Article

Impact of the Vegan Diet on Sperm Quality and Sperm Oxidative Stress Values: A Preliminary Study

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Abstract

Background: Insufficient nutrition and inappropriate diet have been related to many diseases. Although the literature confirms the hypothesis that particular nutritional factors can influence the quality of semen, until today, there are no specific dietary recommendations created for infertile males. Since the male contribution to the fertility of a couple is crucial, it is of high importance to determine the dietary factors that can affect male fertility. **Aim:** The aim of the present study was to evaluate differences in sperm quality parameters, sperm oxidative stress values and sperm acrosome reaction between vegan diet consumers and non-vegans. **Setting and Design:** Prospective study in a University Medical School. **Materials and Methods:** The present study was undertaken to evaluate the sperm quality parameters of vegan diet consumers (10 males who had a strictly vegetable diet with no animal products) and compare them with non-vegans (10 males with no diet restrictions). Semen quality was assessed following the World Health Organization (2010) criteria. Acrosome and DNA integrity has been evaluated using the immunofluorescence technique. **Statistical Analysis:** All variables were analysed by IBM SPSS version 24. Mean differences among groups were compared by Mann–Whitney U-test. **Results:** Obtained results showed that total sperm count (224.7 [117–369] vs. 119.7 [64.8–442.8]; $P = 0.011$) and the percentage of rapid progressively motile sperm were significantly higher in the vegan group compared with the non-vegan group (1 [0–7] vs. 17.5 [15–30]; $P < 0.0001$). Furthermore, the oxidation-reduction potential (0.4 [0.3–0.9] vs. 1.5 [0.6–2.8]; $P < 0.0001$) and the proportion of spermatozoon with DNA damage (14.7 [7–33.5] vs. 8.2 [3–19.5]; $P = 0.05$) were significantly higher in the non-vegan group in comparison to the vegan group. **Conclusions:** Results obtained in this study provide additional evidence about the favourable effect of a plant-based diet on sperm parameters. To confirm our preliminary findings, further studies including larger cohorts are warranted.

Keywords: *Acrosome reaction, diet, oxidation-reduction potential, sperm quality, vegan*

Introduction

Infertility affects about 15% of the world's population; male factors of infertility are responsible for 40%–50% of these cases. Two studies of meta-analysis including more than 40,000 men reported that human sperm count parameters have decreased by approximately 50%–60% worldwide in the last few decades, creating serious concerns about the future of

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human fertility.[1,2] Aside from the genetic, epigenetic or physiologically caused infertility, lifestyle factors such as unhealthy diet, decreased physical activity, smoking and alcohol consumption have been suggested as potential risk factors for poor semen quality.[3]

Insufficient nutrition and inappropriate diet have been related to many diseases, including obesity, cancer, cardiovascular disease and diabetes.[4,5] Although the literature confirms the hypothesis that particular nutritional factors can influence the quality of semen and some of the researchers reported that obesity and overweight decreased sperm parameters and affect couple's fertility,[6,7] until today, there are no specific dietary recommendations created for infertile male. Since the male contribution to the fertility of a couple is crucial, it is of high importance to determine the dietary factors that can affect male fertility.

In the last decade, various studies have reported the beneficial effect of a strict vegetarian diet on general health.[8] Plant-based dietary ingredients have an essential function to defend against the uncontrollable development of reactive oxygen species (ROS).[9]

When we speak about a vegetarian diet, the usual practice is abstinence from the consumption of meat – red meat, poultry, seafood and all other animal flesh. In addition, there are some differences in vegetarian diet such as ovo-vegetarians that consume eggs but not dairy products and lacto-ovo vegetarians that include both dairy and eggs in their diet. Vegans, on the other hand, are very strict vegetarians who consume plant products only and exclude meat, eggs, dairy and animal-derived ingredients in their diet.[10]

Many studies indicate that the health benefits of plant food-based diets, such as vegetarian and vegan diets, could be due to increased antioxidant content, considering decreased intake of saturated fatty acids and increased intake of protein, carotenoids and different forms of fruits that maintain high antioxidant vitamin status.[11,12] ROS may have a significant impact on the plasma membrane of the sperm and sperm functional integrity, leading to decreased sperm motility and viability[13,14] as well as the impaired potential for fertilisation,[15] including capacitation,[16] and acrosome reaction.[17]

Today, <10% of the world population follows a plant-based diet, although this type of diet has positive effects on many metabolic parameters.[18] On the other hand, the role of soy ingredients in male fertility remains unclear, and the main issue is that isoflavones induce oestrogen-like effects on sperm, causing the possible harmful impact on infertility and feminisation in males.[19,20] More attention to this issue was brought by Chavarro *et al*.[21] who reported an inverse correlation between soy food intake and sperm concentration.

Since the published data about the diet and sperm quality are contradictory and there is no concrete comparison between the impact of vegan and non-vegan diets on sperm parameters, this study was designed to investigate possible differences. The aim of the present study was to evaluate the sperm quality parameters of vegan diet consumers and compare them with non-vegans, as well as evaluation of the oxidative stress values and sperm acrosome reaction between these two groups.

Materials and Methods

Ethical statement

Ethical approval of this study was obtained from the local Ethics Committee of the Medical Association of Saarland (reference number: 187/19). Written informed consent was obtained from all the men on the day of semen sample collection. Adherence to the Helsinki Declaration (ethical principles for medical research) was ensured throughout the study process.

Experimental design

A total number of 20 human semen samples were obtained at the Department of Reproductive Medicine, Gynecology and Obstetrics, Homburg (Germany), from individuals volunteering to participate in our study between May and October 2019. Participants in the study mainly were students recruited by personal contacts at the Campus of Saarland University (Homburg). During the abstention period, all the recruited participants committed to refraining from alcohol and were asked to keep their activity level constant. Men were divided into two groups: vegans' participants (N = 10) were males on a strict plant-based diet that included soy and no animal product items for more than 1 year. Non-vegans' participants (N = 10) were males without diet restrictions, who consume animal products and meat on a daily basis. The study included only participants with normal body mass index (BMI). BMI was calculated and the range from 18.5–24.9 kg/m^2 was considered normal.

The inclusion criteria of age for both groups were between 18 and 35 years. When we speak about the number of participants, one of the difficulties was to avoid statistical differences in age between the vegan and non-vegan groups, therefore only participants younger than 35 years, who are not smokers neither alcohol consumers were included in the study. Therefore, even with a sample size of 10 per group, the effect sizes for observed differences with significant comparisons were in the range between 0.6 and 0.9, so the number of

included participants, although small, is acceptable from the scientific side.

In both groups, the exclusion criteria included the following: smoking, coexisting systemic disease, injury or cancer and a history of mumps.

Semen processing

All participants followed a period of 4 days of ejaculatory abstinence. A semen sample was produced on-site by masturbation and collected into a sterile cup. After collection, the sample was liquefied for 20–30 min before analysis at 37°C and maintained throughout the evaluation at the same temperature. Within 1 h of collection, all samples were evaluated. Volume, colour, pH, viscosity, viability by eosin and Mixed Antiglobulin Reaction (MAR) test were processed according to the WHO laboratory manual for the examination of human semen.[22] To avoid the intra-observer variability, each sample was studied at least three times by the same examiner.

Routine sperm analysis including concentration and motility was assessed with the help of a Makler chamber according to the WHO (2010) guidelines. Sperm motility was classified into four groups: rapid progressive (PR-A), progressive (PR-B), non-progressive (NP) and immotile. Morphology was assessed by the Papanicolaou staining method, where at least 200 sperm cells per slide were classified under microscope with a total magnification ×1000 using a high qualitative ×100 non-phase contrast objective under oil immersion and correctly adjusted bright field.

Assessment of reactive oxygen species

The oxidation-reduction potential (ORP) was evaluated using novel galvanostat-based technology – the MiOXSYS System (Aytu BioScience, USA). Briefly, 30 µL of liquefied sperm was applied at room temperature (RT) to the MiOXSYS sensor. When the sample fills the reference cell, the test begins immediately, effectively finishing the electrochemical circuit. The ORP values are presented in millivolts on the screen after a short period of time (mV). Each sample was measured in duplicate. Since the oxidative stress reflects the relationship between spermatozoa (producers of free radicals) and seminal plasma (an antioxidant reservoir), thus raw ORP values (mV) were normalised to sperm concentration (sORP). In addition, sORP values were calculated when the raw ORP value (mV) was divided by the sperm concentration (sperm count $\times 10^6$/mL). sORP was expressed as mV/10^6 sperm/mL.[23]

Acrosome status

Sperm suspensions (20 µl) were spread over SuperFrost Plus slides, air-dried and permeabilised by methanol for 10 min at RT for immunofluorescence detection of the acrosome. Then, sections were washed for 5 min in phosphate-buffered saline (PBS; Sigma-Aldrich, Germany), exposed to blocking buffer (5% bovine serum albumin [Sigma-Aldrich, Germany] in PBS) for 45 min at RT and incubated with fluorescein isothiocyanate-peanut agglutinin (PNA-FITC) (1:50; L7381, Sigma-Aldrich, Germany) for 45 min in the dark. The slides were then rinsed with PBS solution to remove excess stain. Samples were evaluated using an epifluorescent microscope (Olympus BX53) equipped with a differential interference contrast, multiple-fluorescence filter and a digital coloured camera (XC30 3.2 M P).

For the staining pattern, a total of 200 sperm per slide were evaluated and sperm stained with FITC-PNA were categorised as follows: A – in an intact acrosome, the acrosomal region of the sperm head exhibited a uniform apple–green fluorescence, B – only the equatorial part of the acrosome was stained as acrosome-reacted cells or C – the sperm without fluorescence were considered acrosome-reacted cells[24] [Figure 1].

DNA fragmentation

To evaluate possible DNA damage in sperm samples, acridine orange (AO) staining has been performed. The AO assay was performed as previously described by Varghese *et al*.[25] On each slide, the same examiner observed an average of 200 sperm cells. Spermatozoa with green fluorescence were defined to have normal DNA content, while spermatozoa showing an orange to red fluorescence spectrum were defined to have impaired DNA [Figure 2].

Statistical analysis

All variables were analysed by IBM SPSS version 24 (IBM Corp., Armonk, NY, USA). First, all the data was tested for normal distribution. Mean differences among groups were compared by Mann–Whitney U-test. Continuous non-parametric data were reported as median and range. Differences with $P \leq 0.05$ were considered statistically significant.

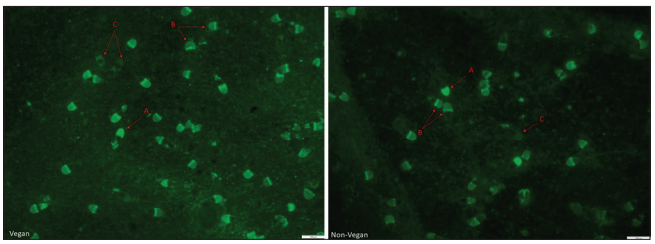

Figure 1: Spermatozoa fluorescence pattern stained with fluorescein isothiocyanate-peanut agglutinin for acrosome status evaluation. (a) Acrosome-intact cells with uniform green fluorescein isothiocyanate-peanut agglutinin fluorescence of acrosome cap. (b) Acrosome-reacted cells that have been stained only by the equatorial part of the acrosome. (c) Acrosome-reacted cells with no staining of acrosome cap

Results

Clinical and laboratory data

According to the guidelines of the World Health Organization, all participants included in the study had normal BMI (22.58 ± 2.59 kg/m^2) and an average abstinence period of 4 days. Vegans have a range from 18 to 31 (median: 23.5) years, while non-vegans have an age range from 21 to 27 (median: 24) years.

Obtained data indicated that there is no significance difference ($P > 0.05$). between vegan and the non-vegan group for the following characteristics: age, BMI, sample volume, sperm viability, and morphology. Although the sperm concentration was higher in the vegan group, the difference was not statistically significant. The main sperm characteristics of vegan and non-vegan participants are presented in Table 1.

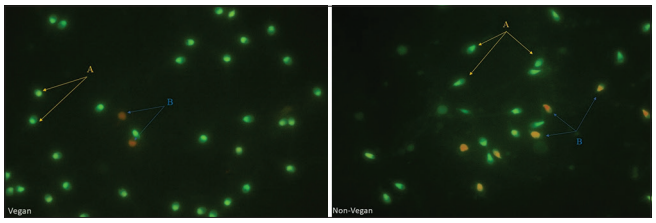

Figure 2: Acridine orange test: Human spermatozoa stained with acridine orange and evaluated under fluorescent microscope. (a) Spermatozoa with normal DNA content. (b) Spermatozoa with damaged DNA

Table 1: Clinical and laboratory data of studied groups

Parameter	Non-vegan (*n*=10)	Vegan (*n*=10)	*P*
Age (years)	24 (21-27)	23.5 (18-31)	0.861
BMI (kg/m^2)	23.8 (20.1-29.9)	22.4 (20.4-28.1)	0.271
Volume (mL)	2.9 (1.6-8.2)	3.6 (2.1-6.1)	0.447
pH	8.70±0.16	8.30±0.13	<0.001
Total sperm count (Mil)	119.7 (64.8-442.8)	224.7 (117-369)	0.011
Concentration (Mil/mL)	43.5 (19-77)	53.5 (41-120)	0.148
Motility (PR + NPR)	51.9±7.57	60.8±6.14	0.011
PR-A (%)	1 (0-7)	17.5 (15-30)	<0.001
PR-B (%)	41.9±5.54	36.3±3.97	0.03
Vitality (%)	67.5±3.95	71.5±4.92	0.10
Morphology (%)	2.8 (2-5)	2.8 (2-5)	0.91
MAR-test (%)	17 (4-58)	7 (0-17)	0.041
ORP (mV)	51.4 (37.1-102.7)	28.2 (15.8-50.4)	0.001
sORP (mV/10^6 sperm/mL)	1.5 (0.6-2.8)	0.4 (0.3-0.9)	<0.001
AO RED (%)	14.7 (7-33.5)	8.2 (3-19.5)	0.05
AR-reacted (%)	72 (25-90)	52.5 (30-80)	0.85

$P \leq 0.05$ was considered statistically significant, Results are presented median, range. *n*=Number of tested cases, BMI=Body mass index, PR=Progressive rapid, NPR=Non-PR, ORP=Oxidation-reduction potential, sORP=Static ORP, AO=Acridine orange, AR=Acrosome reaction, RED=Spermatozoa with impaired DNA, MAR=Mixed antiglobulin reaction

Descriptive statistics between a vegan group and a non-vegan group showed that one of the highest significant differences was obtained in sORP (mV/10^6 sperm/mL) values, where vegans showed a lower range compared to non-vegans (0.4 [0.3–0.9] vs. 1.5 [0.6–2.8], respectively; $P < 0.0001$) [Figure 3].

Total sperm count (Mil) was significantly higher in the vegan group compared to the non-vegan group (224.7 [117–369] vs. 119.7 [64.8–442.8], respectively; $P = 0.011$) [Figure 4]. Furthermore, a total motility (PR-A + PR-B + NP) as well was significantly higher in the vegan group (60 [51–74] vs. 51.5 [42–64], respectively; $P = 0.011$).

Sperm motility classification showed that non-vegans had a lower percentage of rapid motile sperm (PR-A) compared to vegans (1 [0–7] vs. 17.5 [15–30]; $P < 0.0001$) [Figure 5].

Acridine orange test results

Despite the fact that the analysis did not confirm any significant differences between the morphology of these two groups, the AO test showed that the percentage of spermatozoa with DNA denaturation was significantly higher in the non-vegan group compared with the vegan group (14.7 [7–33.5] vs. 8.2 [3–19.5], respectively; $P = 0.05$).

Acrosome reaction test

For the percentage of cells that exhibited intact acrosomes, a significant difference amongst the groups was not observed ($P > 0.05$).

Discussion

In the present study, the sperm quality parameters of vegan diet consumers were evaluated and compared with non-vegans. As far as we know, this is the first research, which includes a comparison of sperm parameters, acrosome reaction, DNA integrity and sperm ORP between these two groups.

The main purpose of the present study was to evaluate the differences of ORP related to vegan and non-vegan diets, and obtained results have shown that both sORP and ORP were significantly lower in the vegan group. Oxidative stress and the influence of ROS on human sperm function and male infertility have been intensively studied in recent years. High ROS levels and oxidative stress have been correlated with sperm DNA damage, impaired fertilisation and embryo development.[14,17] Tremellen[13] reported that subfertile men have lower levels of antioxidants in their semen compared to fertile men. Busetto *et al*.[26] confirm that supplementation of the diet with antioxidants can indeed improve the sperm concentration and motility, including effects on DNA

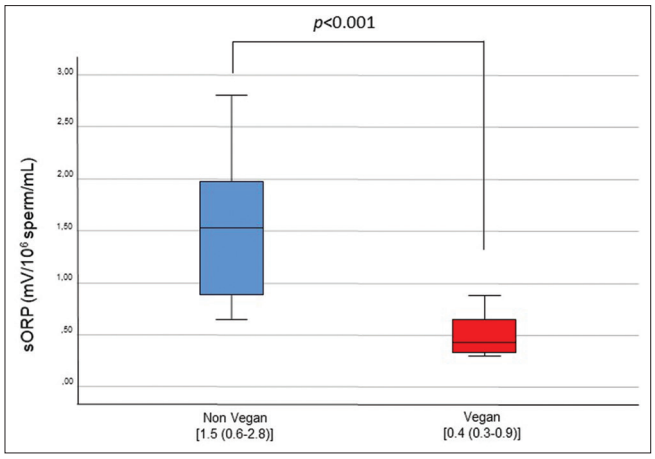

Figure 3: sORP level in vegan and non-vegan groups. Results are presented as median (range)

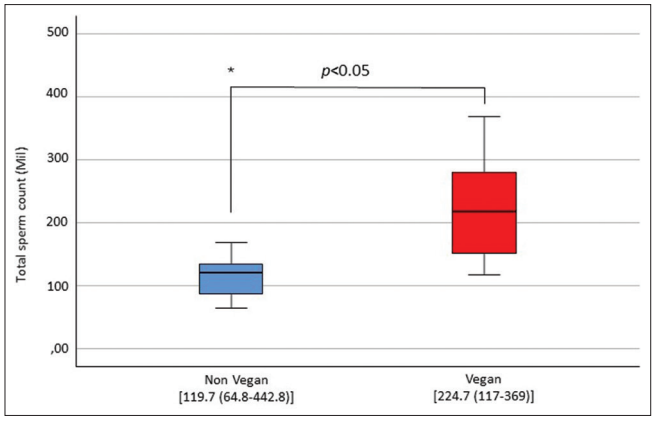

Figure 4: Total sperm count level in vegan and non-vegan groups. Results are presented as median (range)

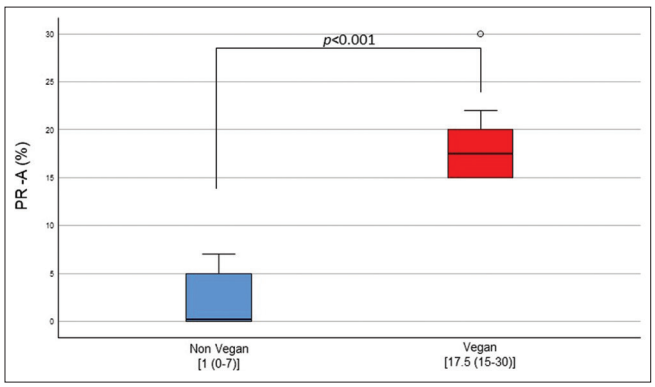

Figure 5: Percentage of rapid motile sperm in vegan and non-vegan groups. Results are presented as median (range)

fragmentation and even pregnancy rate. However, as far as we know, the obtained results in our study are the first report about the impact of a vegan diet on ROS.

Total sperm count in the ejaculate (volume × sperm concentration) between the vegan and non-vegan groups was significantly different, while the difference in volume between these two groups was not significant. Previously, Chavarro *et al*.[27] reported that trans-fatty acids from fried/conventional baked or industrially processed food were inversely associated with the total sperm count. In addition, Karayiannis *et al*.[28] as well reported that low intake of meat and high consumption of fruits, vegetables and whole grains are linked with increased total sperm count. On the other hand, compared to our study in previously cited research, participants were not divided into the vegan and non-vegan groups.

Our results showed that higher consumption of animal-based food was correlated with lower sperm motility. Vegan groups had a significantly higher percentage of rapid progressive sperm as well as a higher percentage of motile (slow progressive + NP) sperm. In literature, several studies have reported that increased intake of Vitamin E, Vitamin C and β-carotene, found in fruits and vegetables, has been linked to a higher percentage of motile sperm in fertile and infertile males.[29-31] In a case–control study of 30 men with reduced semen quality and 31 normozoospermic controls, it was reported that the control group that consumed more lettuce, tomatoes and fruits had a significantly higher percentage of motile sperm compared to infertile cases that consumed more yogurt and meat.[32] In addition, Vessey *et al*.[33] reported that consumption of antioxidants can significantly improve sperm parameters and affect ROS values, what is more they confirmed correlation between ROS levels and sperm motility where compared to our study one of the reasons for better motility in the vegan group might be decreased ORP levels.

One of the parameters which were done in the present study was the evaluation of possible DNA damage in sperm samples where the AO test showed that the percentage of spermatozoa with DNA denaturation was significantly increased in non-vegans. Despite the fact that the *P* value for this parameter was $P = 0.05$, creating a strong conclusion is not possible due to the lack of similar research in the literature.

A further observation from our study demonstrated no significant differences in sperm concentration between those two groups. Braga *et al*.[29] in an observational study of 250 men reported that higher consumption of cereals together with vegetables was positively related to sperm concentration. Furthermore, one of the studies where infertile cases with poor semen quality were analysed, reported that higher intake of meat and processed foods was associated with poor semen concentration.[27] Although in our study, the vegan group generally had higher concentration values, observed results did not show a significant difference in sperm concentration between those two groups, which is in line

with previously published results by Orzylowska *et al*.[10] In addition, compared to the Orzylowska *et al*. study,[10] the reason for different study conclusions might be due to the different sperm WHO criteria used in the study, the difference in age of the participants or because of the different participants' number included in the study. In our research, groups of participants were small but equal, compared to the aforementioned research where the number of participants for vegans was even smaller and between groups strongly unequal: lacto-ovo-vegetarians (N = 26), vegans (N = 5) and non-vegetarians (N = 443). Therefore, one of the study limitations might be the possibility of "type II statistical error" due to the small numbers of compared samples.

Conclusions

Although several studies have shown the favourable effects of the different food categories on male fertility, until today, there are no clear dietary guidelines created for infertile males. For that reason, despite the small sample sizes that could lead to potential confounding, as far as we know, this is the first research, which includes a comparison of sperm parameters, acrosome reaction, DNA integrity and sperm ORP between vegan and non-vegan diet consumers. Results obtained in this study provide additional evidence about the favourable effect of a plant-based diet on sperm parameters. Further, adequately powered studies including larger cohorts are needed in order to unravel the effect of plant-based diet on infertility.

Data availability statement

The data sets used for this study are available with the corresponding author on request.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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