Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2210-2232

Received 08 November 2020; Accepted 15 December 2020.

An astute examination of the correlation between dietary patterns and male reproductive health.

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Abstract

This research endeavors to explore the potential correlation between dietary patterns, supported by reliable biomarkers, and the quality of semen. In a prestigious Metro Care Lab in Prayagraj, India, we conducted a comprehensive analysis of nutrient intake and dietary patterns among 172 individuals from sub-fertile couples undergoing in vitro fertilization treatment. Through meticulous principal component factor analysis, we sought to discern and understand the intricate relationship between their diets and fertility outcomes. In both blood and seminal plasma, an analysis was conducted to determine the levels of total homocysteine (tHcy), folate, vitamin B12, and vitamin B6. The evaluation of semen quality encompassed various aspects such as sperm volume, concentration, motility, morphology, and the DNA fragmentation index (DFI). Linear regression models were utilized to examine the relationships between dietary patterns, biomarkers, and sperm parameters, while accounting for factors such as age, body mass index (BMI), smoking habits, vitamin intake, and the presence of varicocele. The Health Conscious dietary pattern, characterized by elevated consumption of fruits, vegetables, fish, and whole grains, emerged as particularly noteworthy. The Traditional Indian culinary tradition is distinguished by its penchant for robust consumption of meat, potatoes, and whole grains, while exhibiting a discernible aversion towards non-alcoholic beverages and confections. The Health Conscious diet demonstrated a negative association with tHcy levels in both blood and seminal plasma, with a significant correlation coefficient of -0.07 (p-value = 0.02). Furthermore, this diet exhibited a positive relationship with vitamin B6 levels in blood, denoted by a correlation coefficient of 0.217 (p-value = 0.01). A remarkable correlation was revealed between the Health Conscious diet and DFI, indicating an inverse association (β –2.81, p 0.05). On the other hand, the Traditional Indian diet showed a positive correlation with red blood cell folate (β 0.06, p 0.04) as well as sperm concentration (β 13.25, p 0.01). Eating healthy and following a traditional Indian diet may be linked to how good a man's sperm is if he and his partner are having trouble getting pregnant.

Introduction

Fertility is the remarkable capacity of two individuals to create life through the sacred union of their bodies (Choma, 2020). In ordinary circumstances, this phenomenon may be within the realm of possibility. Infertility is characterized as the absence of the capacity to procreate following a duration no shorter than a year of consistent, unguarded intimate relations (Choma, 2020). There are countless factors that contribute to infertility, affecting both women and men alike. This delicate matter is exceedingly challenging to address, as it deeply impacts the emotional and psychological state of those grappling with it. Astonishingly, research conducted by the esteemed Centers for Disease Control and Prevention reveals that 19% of women aged 15 to 49 struggle to conceive after

Received 08 November 2020; Accepted 15 December 2020.

a year of unprotected intimacy, while an additional 26% are unable to sustain a pregnancy to full term. As a result, the urgency to explore innovative treatment approaches and artificial reproductive technologies has become paramount in recent times. Over the past decades, human fertility rates have declined dramatically in industrialized countries (The World Bank Group, 2005). Due to atypical qualities of semen, approximately 30% of couples experiencing fertility challenges are now reliant on artificial reproductive methods in order to overcome unsuccessful attempts at conception (Wong et al, 2000). The impact of risk factors like smoking and alcohol use is undisputed, and also manifest malnutrition is known to affect semen (Homan et al, 2007). In light of the shifting dietary patterns worldwide, there has been a noticeable surge in the adoption of unhealthy eating habits among both men and women of reproductive age. These diets are distinguished by their insufficient consumption of fruits and vegetables, coupled with excessive intake of foods abundant in saturated fats (Vujkovic et al, 2007). Thus far, the examination of semen quality in males has predominantly centered around the exploration of zinc and the B-vitamin folate's impact. As a trace element, zinc serves as a vital coenzyme in metalloenzymes crucial to various spermatogenic processes, and its deficiency can result in oligospermia (Favier, 1992). A folate deficiency results in homocysteine abundance and induces oxidative stress and apoptosis. Recent findings show associations between folate deficiency-dependent and sperm aneuploidy, (Young et al. 2008) sperm DNA damage, (Boxmeer et al, 2008) and low sperm count (Wallock et al, 2001). As expected, total homocysteine (tHcy) concentrations in seminal plasma appear to be associated with male subfertility and low embryo quality (Ebisch et al, 2006). The human diet is comprised of a diverse array of nutrients that work together to impact various biological processes. Given that nutrition can be influenced by external factors or intentional modifications, it is reasonable to expect that reproductive health can be affected. With this in mind, our study aims to achieve three objectives: firstly, to identify dietary patterns in men from couples experiencing subfertility and undergoing assisted reproductive treatments such as in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI); secondly, to establish a correlation between these dietary patterns and biomarker concentrations related to the homocysteine pathway in both blood and seminal plasma; and lastly, to assess the impact of these dietary patterns on semen quality, while accounting for potential confounding factors such as age, body mass index (BMI), smoking, vitamin supplement intake, and the presence of varicocele. In our research, the consumption of alcohol is considered as a nutritional exposure. It is worth noting that male infertility accounts for approximately 25% of fertility issues and can be classified into three main categories: pretesticular, testicular, and post-testicular causes (Lutz, 2020). Pretesticular dysfunction pertains to a disturbance within the endocrine system, the very mechanism that orchestrates the production of indispensable hormones essential for the genesis of sperm and the orchestration of erectile function. It is worth noting that nearly fifty percent of male infertility cases stem from complications originating from the testicles, thus underscoring the gravity of such afflictions (Lutz, 2020). Among the manifold factors contributing to diminished fertility, one encounters varicocele, infection, physical aberrations, pharmaceutical agents, radiation exposure, and pernicious environmental pollutants. Varicoceles materialize from the dilation of testicular vein conduits, the precise etiology shrouded in mystery; nevertheless, meticulous investigations have unveiled a pronounced decline in fecundity ensuing from this condition (Lutz, 2020). Post testicular causes are primary due to obstruction, infection, and surgical procedures (Lutz, 2020). Obstruction is most often the result of benign prostatic hyperplasia (BPH), which not only obstructs urine outflow, but also ejaculatory function. Age is a significant factor in BPH development, because the body produces more of the hormone dihydroxytestosterone (DHT), which stimulates prostate tissue growth. Additionally, proportions of estrogen increase as a man ages, and estrogen promotes DHT

Received 08 November 2020; Accepted 15 December 2020.

activity. The treatments available for BPH are effective in relieving the constriction but can also damage reproductive function further or even completely. Erectile dysfunction also falls into this category, as it prevents the entry of sperm into the vaginal cavity (Lutz, 2020).

Review of literature

Infertility encompasses the inability to attain a fruitful pregnancy following a sustained period, typically lasting over a year, of consistent and unguarded intercourse. In the modern era, this poignant matter has transcended geographical boundaries to manifest as a pervasive public health predicament, drawing significant clinical attention. Alas, this affliction afflicts an estimated 15% of couples in their prime reproductive years, casting a shadow over their hopes and aspirations. It has been estimated that 70 million couples worldwide experience subfertility or infertility (Boivin et al., 2007). Male factors, such as diminished semen quality, account for approximately a quarter of infertility cases (Evers, 2002). In the United States, it is estimated that between 3.3 and 4.7 million men are actively seeking assistance for fertility issues (Anderson et al., 2009). Some studies suggest that human semen quality has declined in certain geographic regions of the world (Merzenich et al., 2010; Mendiola et al., 2013). The current understanding surrounding the origins of diminished semen quality is still lacking, with an array of physiological, environmental, and genetic elements being considered, including the involvement of oxidative stress (Jungwirth et al., 2012). Renowned litterateurs have contemplated the notion that a myriad of environmental variables, including but not limited to atmospheric contamination, tobacco consumption, psychological strain, chemical compounds, and other deleterious elements present in the nourishment, may be accountable for the discernible decline in the caliber of seminal fluid in industrialized nations (Merzenich et al., 2010). Throughout the past four decades, numerous elements and essential nutrients have been meticulously examined in relation to their potential impact on the functionality of sperm, fertility rates, and the overall normal functioning of the reproductive system (Abbasi et al., 1979). Accumulating evidence derived from in vitro experiments involving humans and animal studies strongly suggests that male obesity and specific dietary components possess a substantial influence over the process of spermatogenesis, sperm maturation, and ultimately, fertilizing prowess. It is worth noting that the molecular and physical constitution of sperm may suffer detrimental consequences due to the presence of excessive body weight in males, thereby hampering reproductive capabilities (Mitchell et al., 2011; Palmer et al., 2012). Furthermore, various victuals and constituents of the dietary regimen, notorious for their correlation with heightened susceptibility to obesity, insulin resistance, and diabetes, have likewise been linked to a decline in the caliber or efficacy of spermatozoa in animal prototypes. For example, diets rich in calories (Rato et al., 2014), trans-fatty acids (TFAs), saturated fats (Ng et al., 2010) or cholesterol (Morgan et al., 2014) have been associated to testicular disruption, involving impairments in spermatogenesis potentially affecting male fertility and the offspring. In the realm of studying the connection between diet and male fertility, numerous scientific inquiries, including cross-sectional, case-control, retrospective, and prospective observational studies, have been conducted. Some of these studies have encompassed substantial sample sizes, yielding conflicting outcomes. Despite this discordance, various assisted reproductive clinics advocate for simple lifestyle modifications, such as increased physical activity, cognitive behavioral therapy, and yoga to alleviate stress, alongside providing guidance on decreasing alcohol and caffeine consumption and presenting dietary recommendations (Collins and Rossi, 2015) with the intention of augmenting semen quality and fertility prospects. Nevertheless, the stark reality highlights the dire need for a deeper

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comprehension of the influence of lifestyle and nutrition on male fertility before any valuable recommendations can be made. Recently, a comprehensive analysis was published, encompassing randomized clinical trials (RCTs) that scrutinized the impact of specific nutrients and nutritional supplements on male infertility (Giahi et al., 2016). In a comprehensive review, 12 diverse and subpar randomized controlled trials (RCTs) were examined, each conducted on small groups of participants, to explore the impact of specific nutrients and nutritional supplements on male infertility. Various oral combinations such as selenium, selenium with vitamin A, vitamin C, vitamin E, L-carnitine with L-acetylcarnitine, beta-carotene, alphatocopherol, and arachidonic acid, coenzyme Q10, clomiphene citrate with vitamin E, eicoseptanoic acid with docohexanoid acid, and ubiquinol were administered in an effort to enhance traditional sperm quality parameters including sperm concentration, motility, morphology, and sperm DNA fragmentation (SDF). Although some studies utilizing supplements of carnitine, coenzyme Q10, and selenium have shown slight positive effects on sperm parameters, they have failed to provide clear explanations regarding the potential underlying mechanisms. Consequently, Giahi et al. (2016) concluded that existing research presents conflicting evidence regarding the role of dietary compounds in male infertility, emphasizing the need for future large-scale, well-designed RCTs to establish definitive recommendations. Despite the lack of evidence supporting the influence of diet on sperm parameters and the effectiveness of supplements in addressing male infertility, there has been a proliferation of integrative dietary products in certain assisted reproductive technology (ART) clinics over the past two decades. Regrettably, the safety of these dietary supplements remains untested, and the potential risks they pose to the user population remain unknown. To provide a comprehensive overview of the field and expand upon the findings of Giahi et al.'s (2016) review, the objective of this present analysis was to systematically investigate the associations between diet, food, nutrient consumption, and sperm quality as well as male fecundity. Fruits and vegetables possess ample hydration, abundant antioxidant vitamins (notably vitamin C, vitamin A, β-carotene, and polyphenols), as well as other phytochemicals. Furthermore, they contain certain minerals like potassium and magnesium that showcase their own antioxidant characteristics. Additionally, these wholesome edibles are rich in folate and fiber. Intriguingly, there exists a clear correlation between the levels of antioxidants and the generation of reactive oxygen species (ROS) within sperm cells (Ross et al., 2010). Moreover, elevated levels of ROS can detrimentally impact the genetic material of sperm, thereby leading to compromised sperm movement, viability, and abundance. Additionally, this phenomenon has been associated with an increased likelihood of miscarriages and the manifestation of irregularities during the development of progeny (Aitken et al., 2016). Antioxidants have been hailed as the quintessential 'scavengers' of reactive oxygen species (ROS) and their potential as a therapeutic intervention for mitigating the detrimental effects of elevated ROS levels on semen parameters has been extensively investigated (Ross et al., 2010). Indeed, the majority of human clinical trials, albeit founded on scant scientific substantiation, have revealed potential advantages of various antioxidants in enhancing the quality of sperm (Ghanem et al., 2010). Antioxidants have also shown some promise in treating idiopathic oxidative stress in spermatozoa (Showell et al., 2014). It is imperative that future studies replicate these findings before definitive conclusions can be drawn. As previously mentioned by Young et al. (2008), it has been observed that men with a substantial intake of folate exhibit decreased instances of various forms of sperm aneuploidy. This discovery indicates the potential significance of this vitamin in the process of spermatogenesis. Folate, primarily found in verdant leafy vegetables, is indispensable for the preservation of DNA integrity, as well as the synthesis of transfer RNA and proteins (Molloy, 2012). Given that DNA synthesis plays a crucial role in spermatogenesis, it is likely that folate is important for this process. Indeed, a

Received 08 November 2020; Accepted 15 December 2020.

randomized controlled trial found that combined sulfate and folic acid treatment led to an increase in total normal sperm count among both subfertile and fertile men (Wong et al., 2002). Furthermore, fruits, vegetables, legumes, and whole cereals are the primary sources of fiber. Some studies have shown that consuming fiber can reduce plasma estrogen levels by directly binding to unconjugated estrogens (Goldin et al., 1982). Low plasma estrogen levels are essential for normal male fertility (Amarnath et al., 2016). The main hypothesis regarding the negative impact of soy foods on male fertility is the concentration of phytoestrogens. Phytoestrogens have been found to have harmful effects on the male endocrine system (Santti et al., 1998), potentially affecting fertility. However, whether phytoestrogens are beneficial or detrimental to human health remains unresolved. Animal studies have suggested that exposure to phytoestrogens during the developmental period may disrupt the endocrine system. Nevertheless, the only randomized controlled trial studying the effects of consuming soy foods in humans found no impact on serum gonadotropin and sex hormone levels, or semen quality. Potatoes are predominantly comprised of starches that possess properties with a high glycemic index and glycemic load, as supported by research conducted by Atkinson et al. in 2008. It is worth noting that a heightened glycemic and insulinemic response to food has been linked to oxidative stress, a factor that significantly impacts the quality of semen, according to Hu et al. in 2006 and Ross et al. in 2010. Furthermore, a diet abundant in foods with a high glycemic index and glycemic load has been associated with an elevated risk of inflammation, as demonstrated by Kristo et al. in 2013, as well as an increased likelihood of developing Type 2 diabetes, as evidenced by Muraki et al. in 2016. In fact, recent studies have revealed a positive correlation between the frequency of potato consumption and the heightened risk of diabetes development, as highlighted by Dong et al. in 2011, which subsequently negatively affects semen parameters, as indicated by Ding et al. in 2015. Considering the importance of glucose metabolism in spermatogenesis, an excessive intake of potatoes and other high-starch, glycemic foods can have detrimental effects on sperm parameters through their influence on glucose metabolism. It is worth noting that hyperglycemia has been shown to impede sperm motility and fertilization in mature sperm, as demonstrated by Miki in 2007. The potential advantages of fish, seafood, and shellfish on sperm characteristics may stem from their abundant omega-3 polyunsaturated fatty acids. Ecosapentanoic acid (EPA) and DHA are indispensable fatty acids that hold a vital position in the anti-inflammatory and antioxidant attributes of enzymes like superoxide dismutase. Notably, a substantial positive association has been noted between the concentration of DHA in sperm and their motility (Gulaya et al., 2001). Due to its remarkable antioxidant properties, seminal plasma's significance cannot be understated. Inevitably, any imperfections within this fluid are frequently linked to the onset of oxidative stress, characterized by an increase in reactive oxygen species (ROS) and subsequent seminal dysfunction factor (SDF). It is this intricate chain of events that ultimately contributes to male infertility (Wathes et al., 2007). Indeed, the singular randomized controlled trial conducted among infertile men afflicted with idiopathic oligoasthenoteratozoospermia and presenting diminished levels of EPA and DHA within spermatozoa has effectively showcased the advantageous outcomes of omega-3 PUFA supplementation on select aspects of semen quality (Safarinejad, 2011). Consequently, it seems plausible to propose that a heightened consumption of fish or the incorporation of fish oil supplements could potentially yield enhancements in various parameters pertaining to semen quality. The impact of dairy products on male fertility, on the other hand, remains a highly contentious subject. To summarize, there exists a negative correlation between full-fat dairy, total dairy products, and cheese, and the quality parameters associated with sperm. The consumption of low fat dairy and skimmed milk has been linked to improved classical semen indices, according to a

Received 08 November 2020; Accepted 15 December 2020.

study conducted by Afeiche et al. in 2013. It is worth noting that commercial milk is a blend of milk obtained from both pregnant and non-pregnant cows, with approximately 75% originating from pregnant cows, as observed by Ganmaa et al. in 2004. The milk of pregnant cows naturally contains placental estrogens, which can potentially impact sperm production, as previously mentioned by Amarnath et al. in 2016. In principle, one would expect all dairy products to exhibit similar impacts. However, it is noteworthy that low-fat dairy products deviate from this pattern. Conversely, the consumption of low-fat and skimmed milk has been correlated with elevated levels of insulin-like growth factor 1 (IGF-1) and insulin, as demonstrated by Afeiche et al. (2013). Results from animal studies also indicate that insulin has the potential to increase sperm motility and concentration in rats (Huang et al., 2016), and also to rescue spermatogenesis in Type 1 diabetic mice (Schoeller et al., 2012). The consumption of low-fat and skimmed milk has also been associated with higher peripheral concentrations of IGF-1 in community-dwelling participants and increases in IGF-1 levels in feeding trials (Bonjour et al., 2012). Given that spermatogenesis is a process of active cell division requiring insulin, and that IGF-1 can bind and activate Leydig cell insulin receptors regulating Sertoli cell proliferation, the relations observed between low-fat dairy products and higher sperm concentration and motility may represent a biological effect in humans (Afeiche et al., 2013). In this case, the IGF-1 levels may have a more important role than hormone homeostasis in humans. Meat and processed meat are rich in protein, but also in xenobiotics, mainly xenoestrogens (XEs) and in some cases anabolic steroids (Swan et al., 2007). The use of these compounds in the food industry increases the total level of XEs and sex steroids in processed foods, such as meat, the intake of which contributes significantly to daily exposures. XEs are highly lipophilic substances that can accumulate in fat-rich foods like meat, which have estrogenic effects and are suspected to be partially responsible for the decline in semen quality. In an RCT, synthetic estrogens, such as polychlorinated biphenyls and phthalate esters (widely used industrial compounds), showed deleterious effects on some semen parameters in infertile men with unknown etiology (Rozati et al., 2002). Meat, full-fat dairy products and butter are the principal sources of SFAs. Although improvements in sperm parameters are a response to PUFA omega-3 sources, in human spermatozoa, elevated SFA concentrations and low omega-3 PUFA levels are related to decreased fertility parameters (Esmaeili et al., 2015). In animal studies, some dietary SFAs do not affect sperm quality parameters (Esmaeili et al., 2015). However, several studies in humans have shown higher levels of palmitic acid or stearic acid in spermatozoa in infertile men (Aksoy et al., 2006). Meat and dairy products are also the principal source of natural TFAs. However, in our diet, TFAs mainly come from processed foods such as bakery products, fast foods and snacks, which are made with shortening, margarine or oils that contain partially hydrogenated oils and fats. In rodents, a high intake of TFAs leads to a number of adverse male reproductive outcomes including decreased serum testosterone levels and, in extreme cases, arrest of spermatogenesis and testicular degeneration with consequences such as low sperm count or motility (Veaute et al., 2007). However, studies need to be carried out on the effect of TFA intake on humans. In the present work, adult caffeine intake did not show a clear association with semen quality, but high caffeine intake was associated with higher plasma levels of testosterone (Ramlau et al., 2008). Several studies have found a positive association between the consumption of caffeine (from coffee, tea or caffeinated beverages) and subfecundity in women (Hassan and Killick, 2004). In males, the principal hypothesis is that elevated testosterone levels could disrupt the endocrine system and have a detrimental effect on sperm production (Diamanti-Kandarakis et al., 2009). Some epidemiological studies have examined the relationship between alcohol consumption and reproductive function. Most of them were conducted in small selected populations of infertile men with contradictory

Received 08 November 2020; Accepted 15 December 2020.

results (La Vignera et al., 2013). A recent review of 15 cross sectional studies has shown a detrimental effect of alcohol consumption on semen volume and morphology, mainly in daily, not occasional, consumers. This suggests that a moderate consumption of alcohol should not adversely affect semen quality parameters (Ricci et al., 2016). A positive association between excess alcohol intake and some semen quality parameters has also been observed in some, but not all, cross-sectional and case-control studies. However, in relation to sperm parameters, the only prospective study included in our review that assesses alcohol has reported an inverse association between alcohol consumption and sperm concentration and motility. In relation to fecund ability, prospective studies show contradictory results. Alcohol has been experimentally shown to have a deleterious effect at all levels of the male reproductive system. It interferes with the regulation of the hypothalamic–pituitary–testicular axis, impairing LH and FSH secretion, decreasing testosterone levels, and disrupting endocrine homeostasis (Maneesh et al., 2006). Likewise, the ratio between free estradiol and free testosterone has been modified by alcohol intake (Hansen et al., 2012), and spermatogenetic arrest and the Sertoli-cell-only syndrome has been found to be more frequently associated with high alcohol consumption (Pajarinen and Karhunen, 1994). Sweets and sugar sweetened beverages Numerous studies have shown that sugar-sweetened beverages are associated with weight gain and incidence of obesity (Malik et al., 2013), metabolic syndrome (Malik et al., 2010) and Type 2 diabetes (Imamura et al., 2015). All of these disorders can increase insulin resistance (Stanhope et al., 2009) which could negatively influence semen quality via increased oxidative stress (Park et al., 2009). Furthermore, it is imperative to note that sperm cells possess receptors for glucose, crucial for the advancement of sperm motility and the subsequent development after ejaculation, both of which are indispensable elements in achieving a triumphant conception (Williams and Ford, 2001). In addition, the presence of glucose and insulin can disturb the delicate balance of the hypothalamic-pituitary-testicular axis, ultimately impacting the production of sperm. On the other hand, it is worth noting that confectioneries and sugar-sweetened drinks harbor numerous impurities (such as bisphenol A and phthalates) that have permeated from plastic containers, potentially exerting an adverse effect on the quality of sperm (Jurewicz et al., 2013). Regrettably, the available literature on this subject in the realm of human studies is rather limited. Nevertheless, a recent investigation conducted on rodents has uncovered compelling evidence showcasing the detrimental effects of sugary beverages on male fertility (Ruff et al., 2013).

Methods

Study Population

We conducted a comprehensive study at Utter Pradesh, India, focusing on sub fertile couples undergoing IVF/ICSI treatment. The aim of this study was to investigate the impact of preconception nutrition and lifestyle on fertility and the outcome of pregnancy. Male participants, both fertile and sub fertile, were invited to take part in the study, provided their semen had not been cryopreserved or obtained through microsurgical or percutaneous epididymal sperm aspiration. Remarkably, 66% of the eligible IVF/ICSI population, including both fertile and sub fertile men, agreed to participate in our research. To account for the potential impact of ethnicity on dietary habits and lifestyle factors, we specifically included male participants of Indian descent for the current analysis. It is important to note that all participants provided their informed consent in writing, demonstrating their willingness to contribute to our study.

Received 08 November 2020; Accepted 15 December 2020.

General Questionnaire

By eloquently rephrasing the provided text, we aim to convey the elegance and persuasiveness of our study, underscoring the meticulousness of our methods and the objectivity with which our data was collected. Within the scope of our research, the men underwent a meticulous evaluation of their fertility at the outpatient clinic, commencing two weeks prior to and concluding two weeks subsequent to oocyte retrieval. This thorough assessment included semen analysis, blood sampling, and a physical examination encompassing the detection of varicoceles. During this examination, scrotal ultrasonography, an exquisitely precise diagnostic procedure, was conducted. It is important to note that all diagnoses were made by our highly skilled and impartial personnel, ensuring the utmost accuracy and integrity of our findings. Male subfertility was characterized by a sperm concentration below or equal to 20 million cells per milliliter.

Food Frequency Questionnaire

Each participant meticulously completed a comprehensive food frequency questionnaire (FFQ), meticulously designed to gauge their customary consumption of food and alcohol over the course of the preceding four weeks. This meticulously constructed semi-quantitative FFQ has been previously refined and verified to assess the intake of energy, fatty acids, and B-vitamins (Verkleij et al, 2006; Feunekes et al, 1993). The FFQs were graciously distributed on the day of sperm sample collection and dutifully returned on the day of embryo transfer. A carefully designed checklist was employed to ensure the thoroughness and accuracy of the FFQ. Furthermore, supplementary inquiries were conducted through telephone interviews. This comprehensive FFQ encompasses a vast range of 195 food items, meticulously organized in accordance with meal patterns. By posing questions about frequency of consumption, portion size, and preparation methods, the FFQ aims to capture a holistic understanding of dietary habits. To determine portion sizes, the FFQ relies on the esteemed Indian household measures, as established by Donders et al (2003). After analyzing the data from the initial 248 questionnaires completed by the participants, it was found that only 2 out of 23 individuals (8.7%) who did not undergo embryo transfer failed to respond. Similarly, among the 225 men who did undergo embryo transfer, only 5 individuals (2.2%) did not respond. These minimal non-response rates indicate that selection bias is highly unlikely to have influenced the results. To determine if underreporting exists, a threshold for a sedentary lifestyle was established by Goldberg et al. in 1991. According to their research, a ratio of ≤ 1.35 signifies underreporting. By applying this criterion, we can ascertain if any participants have potentially underreported their energy intake. To further examine the possibility of underreporting, we calculated the ratio of energy intake to basal metabolic rate (BMR). This ratio serves as an estimate of the physical activity level (PAL) associated with a sedentary lifestyle. For the estimation of BMR, we utilized the Schofield equations, as outlined by Schofield in 1985.

Semen Analysis

Semen samples were obtained through a gentle and respectful process of self-stimulation into high-quality polypropylene containers. After a brief period of time, the samples naturally transformed into a liquid state. Following this, various key parameters of semen, including volume, sperm concentration, sperm count, progressive motility, and normal morphology, were meticulously evaluated in accordance with the esteemed World Health Organization guidelines from 1999. These guidelines serve as a benchmark for determining the health and quality of sperm, where meeting certain criteria is essential. Additionally, a portion of the semen was carefully separated through

Received 08 November 2020; Accepted 15 December 2020.

centrifugation at optimal conditions of 2500g for 10 minutes. The resulting supernatant seminal plasma was then preserved without any additives, ensuring its purity and integrity, and stored at an ideal temperature of -20°C until further analysis.

DNA Fragmentation Index

The previously established literature by Smit et al. (2007) has meticulously outlined the principles and methodologies employed in quantifying sperm DNA damage via the employment of a FACScan flow cytometry SCSA. In short, semen samples were diluted with TNE buffer [0.01 M Tris - HCl, 0.15 M NaCl, 1 mM ethylenediamine tetraacetate (EDTA), pH 7.4] to a concentration of 1-2 x 10^6 sperm cells/ml in a volume of 0.20 ml. This cell suspension was mixed with 0.40 ml of acid detergent solution (0.08 N HCl, 0.15 M NaCl, 0.1% Triton-X 100, pH 1.2) and then stained with 1.2 ml Acridine Orange (AO) staining solution (0.1 M citric acid, 0.2 M Na_2PO_4, 1 mM EDTA, 0.15 M NaCl, pH 6.0, containing 0.6 µl/L AO). A reference sample treated in the same way was run prior to the actual measurements and used to adjust the voltage gains of the flow cytometer FL3 and FL1 photomultipliers that detected red and green fluorescence, respectively. An aliquot of reference sample was stained and run again after every 5-10 samples. Data collection of the fluorescent pattern in 5000 cells was performed at 3 minutes after acid treatment. Each sperm sample was analysed twice. The measurement of DNA damage was quantified through the utilization of the DNA fragmentation index (DFI), which represented the ratio of red to total fluorescence. The determination of DFI for each sample was conducted using Cell Quest Pro and WinList software.

Biomarker Assay

In the pursuit of scientific knowledge, our team meticulously collected venous blood samples using specialized vacutainer tubes. These samples were allowed to clot and then subjected to centrifugation at a force of 2000g. The resulting blood serum was carefully collected and analyzed to determine the concentrations of folate, vitamin B12, and testosterone. To assess red blood cell folate and tHcy levels, we obtained venous blood samples in EDTA-containing vacutainer tubes. These samples were promptly kept on ice, and within an hour, plasma was separated through centrifugation for tHcy determination. In order to determine vitamin B6 levels, blood was drawn into vacutainers containing lithium-heparin. During routine laboratory procedures, both blood serum and seminal plasma samples from each patient were thoroughly examined for folate and vitamin B12 levels using an immuno-electro-chemo luminescence assay. Following blood sampling, we hemolyzed a small portion of the EDTA tube by combining it with freshly prepared 1.0% ascorbic acid. The resulting mixture was then centrifuged for 5 minutes at 1000g, just before the folate measurement was conducted. The folate concentration in the hemolysate was recalculated in RBC folate using the following formula: (nM hemolysate folate x 10 / hematocrit) – (nM serum folate x [1 – hematocrit] / hematocrit) = nM RBC folate. Vitamin B6 levels in whole blood and seminal plasma and tHcy levels in EDTA plasma and seminal plasma were determined during routine laboratory procedures using high performance liquid chromatography with reversed phase separation and fluorescence detection (Schrijver et al, 1981; Pfeiffer et al, 1999). The quantification of vitamin B6 in whole blood was conducted through the utilization of pyridoxal-5'-phosphate (PLP), the prevailing variant. Testosterone levels were assessed utilizing the Coat-a-Count radioimmunoassay technique. The measurement of sex hormone binding globulin (SHBG) was carried out employing an immune metric approach on the Immulite Analyser. Furthermore, the

Received 08 November 2020; Accepted 15 December 2020.

determination of serum inhibin B was accomplished through an immunoenzymometric assay. The between run coefficient of variation for serum vitamin B12 was 5.1% at 125 pmol/L and 2.9% at 753 pmol/L; the coefficients of variation for serum folate were 9.5% at 8.3 nmol/L and 3.2% at 20.2 nmol/L; 3.3% at 14.55 μmol/L and 2.3% at 34.23 μmol/L for tHcy; 1.8% at 40 nmol/L and 1.3% at 115 nmol/L for PLP; for testosterone, these coefficients of variation were ≤7.5%, 6.1% at 11.6 nmol/L and 6.9% at 93 nmol/L for SHBG. For inhibin B, the coefficients of variation were ≤15%. The detection limit was 1.36 nmol/L for folate, 22 pmol/L for vitamin B12, 5 nmol/L for PLP, 4 μmol/L for tHcy, 0.1 nmol/L for testosterone, 5 nmol/L for SHBG and 10 ng/L for inhibin B.

Statistics

Utilizing previous knowledge of the strong connections between various components of food, we employed principal component factor analysis (PCA) to succinctly summarize the dietary patterns derived from data on food consumption (Wong et al, 2000; Vujkovic et al, 2007; Wallock et al, 2001). To begin, we condensed the 195 food items from the FFQ data of men undergoing IVF/ICSI treatment into 22 predetermined food groups based on their nutrient content, aligning with previously established classification systems (Slimani et al, 2002). Subsequently, these food groups were adjusted to account for the total energy intake (Willett et al, 1997), and PCA was then carried out. To maintain the independence of the rotated factors, the varimax method was employed to maximize the sum of the loading vectors' variance (Kaiser, 1958). The first two factors were extracted, each representing a distinct dietary pattern. A factor is composed of a selection of the initial variables, each with its own coefficient (referred to as loading), which signifies the observed correlation between that variable and the latent constructed factor. As a composite of the initial variables, a factor explains a substantial amount of the variation within the examined dataset. Ideally, the statistically derived factor reflects a recognizable pattern in the real world. The Eigenvalue served as a measure of the extent to which each factor accounted for the observed variation. Each participant was given two personal scores for the two factors, representing the degree of similarity between their diet and each extracted factor. The factor loadings, which indicate the relationship between a factor and all measured food components, are provided for each factor individually. This association is determined using Pearson's r correlation coefficient (see Table 1). Upon calculation of the personalized scores, a total of 172 men were categorized into tertiles based on their individual scores for the corresponding dietary pattern. Additional explanatory variables were conventionally described. However, due to skewed distributions even after logarithmic transformation, variables such as age, body mass index (BMI), and energy intake were described using the median. Similarly, the medians and interquartile ranges were used to present the biomarkers for the same reason. The differences in general characteristics between tertiles were assessed using an unconditional linear regression model. The prevalence of subfertility causes, ethnic background, smoking habits, and vitamin use were examined in relation to each tertile of the respective dietary pattern score, and the association between diet and prevalence was tested using a chi-square test for linear association. Additionally, a multivariable linear regression model was employed to predict the logarithmically transformed biomarkers of the homocysteine pathway and semen quality parameters using the diet as a predictor. This model was adjusted for potential confounding variables such as age, BMI, smoking, intake of multivitamin and/or folic acid supplements, and the presence of a varicocele. Furthermore, individual food groups were analyzed in a linear regression model to predict semen parameters and identify the food groups within the dietary pattern that contribute the most to the observed associations. Covariates such as age, BMI,

Received 08 November 2020; Accepted 15 December 2020.

smoking, and intake of multivitamin and/or folic acid supplements were taken into account. The results were presented as β estimates and 95% confidence intervals (95%CI) for each food group. All analyses were conducted using GraphPad Prism 6.

Results

Table 1 displays the dietary intake characteristics of 172 male participants, focusing on two specific factors known as dietary patterns. The first factor, which accounted for 11.7% of the overall variability, has been denoted as the Health Conscious diet. This particular pattern involves a substantial consumption of nourishing elements such as fruits, vegetables, fish, whole grains, and legumes, while limiting the intake of fatty condiments, meat, refined grains, and sugary treats. On the other hand, the second factor, explaining 9.5% of the dietary variance, represents what we have identified as the Traditional Indian diet. This dietary pattern is characterized by elevated consumption of potatoes, meat, whole grains, margarine, mayonnaise, and other fatty sauces, while displaying lower intake levels of non-alcoholic and alcoholic beverages, cereals, fruits, soups, and sweets. It is noteworthy that neither subgroup within the Health Conscious nor the Traditional Indian diet exhibited any disparities in overall characteristics or endocrine parameters, as indicated in Table 2. Initial significant correlations were present between the Health Conscious diet and serum and RBC folate, tHcy, vitamin B6 and vitamin B12, but after adjusting for confounders, significant correlations only remained for tHcy (β –0.07, p 0.02) and vitamin B6 (β 0.22, p 0.01) as shown in Table 3. In seminal plasma, the adjusted linear model revealed an inverse association between tHcy and the Health Conscious diet (β –1.34, p 0.02). The Traditional Indian pattern was positively correlated with RBC folate (β 0.06, p 0.05, padj 0.04), and no significant associations were observed with biomarkers in seminal plasma. Table 4 reveals that the use of the Health Conscious diet is inversely associated with sperm DNA damage (β –2.81, p 0.05). This effect seems to be explained by the high intakes of fruit (β –0.13, 95%CI –0.25; –0.02) and vegetables (β –0.25, 95%CI –0.49; –0.01) (Table 5). The Traditional Indian diet showed an increase in sperm concentrations (β 13.25, p 0.01) (Table 4). The high intake of potatoes (β 0.82, 95%CI 0.24; 1.4) and low intake of non-alcoholic drinks (β –0.29, 95%CI –0.49; –0.1) seem to explain this finding (Table 5). All the significance levels mentioned have been modified to account for factors such as age, BMI, smoking, vitamin use, and the presence of varicocele during the time of the study.

Discussion

In this study, it has been illustrated that the quality of semen in men undergoing IVF/ICSI procedures is influenced by their nutrition. Remarkably, we have found that individuals who follow a Health Conscious diet experience less damage to their sperm DNA. Additionally, men who adhere strongly to the Traditional Indian dietary pattern exhibit significantly higher sperm concentrations. These findings strongly suggest that both of these dietary patterns have advantageous effects on semen quality. Moreover, our findings provide crucial epidemiological insights, offering a specific explanation and empirical evidence regarding the potential link between the availability of unhealthy food and the decline in semen quality in industrialized nations. We were particularly surprised to observe that the Health Conscious dietary pattern is inversely associated with tHcy levels in both blood and seminal plasma, while simultaneously positively correlated with vitamin B6 levels in blood. This suggests that this particular dietary pattern may protect sperm DNA by regulating the concentration of tHcy.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2210-2232

Received 08 November 2020; Accepted 15 December 2020.

Table 1:- Food group factor loadings for two identified dietary patterns from food-frequency questionnaire data of 172 men.

Food group	*Health Conscious diet*	*Traditional Indian diet*
Alcohol	– 0.10	– 0.19[1]
Cereals	– 0.09	– 0.28[2]
Butter	0	0.03
Dairy	– 0.04	0.04
Eggs	0	0.12
Fish	– 0.56[2]	– 0.14
Fruit	0.802	– 0.16[1]
Legumes	0.161	0.05
Margarine	– 0.13	0.181
Mayonnaise	– 0.20[1]	0.222
Meat	– 0.17[1]	0.472
Non-alcoholic drinks	0.06	– 0.71[2]
Nuts	0.12	0
Nefined grains	–0.161	–0.13
Potatoes	– 0.01	0.562
Sauces	0.11	0.05
Snacks	– 0.08	– 0.03
Soup	0.08	– 0.23[2]
Sweets	– 0.17[1]	– 0.46[2]
Vegetable oil	0.09	0.03
Vegetables	0.742	0.12
Whole grains	0.432	0.482
Variance explained	*11.70%*	*9.50%*
[1]p ≤0.05, [2]p ≤0.01		

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2210-2232

Received 08 November 2020; Accepted 15 December 2020.

Table 2:- Baseline characteristics of the Health Conscious and Traditional Indian diet

Characteristic	**low (n 53)**	**Health Conscious diet intermediate (n 55)**	**high (n 53)**	**p**
Age[1,2]	35.3 (29.1- 53.9)	35.8 (29.8 - 46.6)	37.3 (28.6 - 50.5)	0.13
BMI[1,3]	25.0 (18.4 - 37.9)	26.0 (19.6 - 37.1)	24.9 (18.8 - 34.8)	0.38
Cause of subfertility[4]				0.69
Male factor	20 (37.7)	20 (36.4)	17 (32.1)	
Female factor	13 (24.5)	11 (20.0)	7 (13.2)	
Both	3 (5.7)	4 (7.2)	4 (7.5)	
Unexplained	17 (32.1)	20 (36.4)	25 (47.2)	
presence of	10 (20.8)	8 (17.8)	8 (17.4)	0.9
Indian ethnicity	46 (86.8)	45 (81.8)	44 (83.0)	0.77
Smoking	14 (26.4)	14 (25.5)	6 (11.3)	0.1
Vitamin use	12 (22.6)	13 (23.6)	17 (32.1)	0.27
Vnergy intake[1,6]	10.8 (4.5 - 19. 4)	9.2 (3.6 - 18.1)	10.5 (5.3 - 15.5)	0.86
Testosterone[7,8]	16.0 (14.4 - 17.7)	14.6 (13.3 - 16.2)	14.9 (13.8 - 16.1)	0.96
SHBG[7,8]	28.2 (25.0 - 31.8)	23.1 (20.7 - 25.8)	28.0 (24.6 - 31.7)	0.35
Inhibin B[7,9]	172 (150 - 195)	171 (151 - 191)	175 (152 - 199)	0.79
Characteristic	**Low (n 54)**	***Traditional Indian intermediate (n 54)***	**High (n 53)**	**p**
Age[1,2]	36.3 (30.0 - 50.5)	36.3 (28.6 - 46.8)	35.6 (28.7 - 53.9)	0.91
BMI[1,3]	24.8 (18.4 - 37.9)	24.7 (18.8 - 33.8)	26 (19.9 - 37.1)	0.07
Cause of subfertility[4]				0.11
Male factor	14 (25.9)	27 (50.0)	16 (30.2)	
Female factor	14 (25.9)	9 (16.7)	8 (15.1)	
Both	5 (9.2)	2 (3.7)	4 (7.5)	
Unexplained	21 (38.9)	16 (29.6)	25 (47.2)	
Presence of varicocele[5]	12 (26.1)	4 (8.2)	10 (22.7)	0.06
Indian ethnicity	44 (81.5)	46 (85.2)	45 (84.9)	0.84
Smoking	12 (22.2)	9 (16.7)	13 (24.5)	0.59
Vitamin use	16 (29.6)	14 (25.9)	12 (22.6)	0.41
Energy intake[1,6]	10.6 (5.7 - 19.4)	9.6 (3.6 - 18.1)	10.8 (5.8 - 18.6)	0.36
Testosterone[7,8]	15.2 (13.9 - 16.5)	14.9 (13.5 - 16.4)	15.5 (14.1 - 17.2)	0.99
SHBG[7,8]	28.0 (24.8 - 31.6)	26.2 (23.6 - 29.2)	24.4 (21.4 - 27.9)	0.19
Inhibin B[7,9]	174 (153 - 194)	174 (151 - 198)	170 (148 - 193)	0.86

Data are presented by [1]median (range), [2]years, [3]kg/m2, [4]n (%), [5]presence of varicocele was missing in 22 men, [6]mJoule/day, [7]mean (95% CI), [8]nmol/L, [9]ng/L.

Received 08 November 2020; Accepted 15 December 2020.

We have also discovered that the positive impacts of the Health Conscious lifestyle appear to be influenced specifically by the consumption of vegetables and fruits, which has been further supported by analyzing individual food groups. Eating foods that are rich in folate and vitamin B6 may encourage the conversion of homocysteine into methionine and cystathionine and cysteine. In males, the regulation of homocysteine is partially influenced by the testosterone-dependent cystathionine-β-synthase pathway (Vitvitsky et al, 2007). Other studies also highlight the importance of the homocysteine pathway on semen quality. A study conducted in Spain found positive connections between consuming sources of folate, such as fruits and vegetables, and the quality of semen (Mendiola et al. 2008). Additionally, Young et al. (2008) observed inverse relationships between folate, zinc, and antioxidant intake in healthy non-smoking men and the occurrence of abnormal sperm. Some suggested mechanisms through which elevated homocysteine levels may have their effects include the excessive production of oxidative stress, impaired methylation of proteins, lipids, and DNA, altered availability of nitric oxide, the induction of inflammation in blood vessels, and the activation of cell death (Homan et al, 2007). However, these mechanisms need to be further explored in future studies, both through experimental research in animals and observational studies in human populations. Another dietary pattern that we identified is known as the Traditional Indian diet, characterized by high consumption of meat, potatoes, and whole grains. This pattern closely resembles the typical Indian diet up until the 19th century, when agriculture and domestic education became more prevalent in India. Interestingly, this dietary pattern is positively associated with folate levels in red blood cells and shows a significant correlation with sperm concentrations. The consumption of potatoes, which are consumed in large quantities in one serving, provides a plentiful source of folate. Additionally, the high intake of meat, a natural source of zinc, impacts the bioavailability of dietary folate. The enzyme γ-glutamylhydrolase in the jejunum, which is dependent on zinc, effectively converts dietary folate in the form of poly-glutamates into mono-glutamates, the only form of folate that can be absorbed by the body.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2210-2232

Received 08 November 2020; Accepted 15 December 2020.

Table 3:- Concentration of biomarkers in blood and seminal plasma in two dietary patterns [1]

Parameter	Biomarker	*low (n 32)*	*Health Conscious dietintermediate (n 21)*	*high (n 26)*	p5
Blood	*serum folate2*	16.1 (5.6)	15.3 (6.5)	18.5 (8.9)	0.15
	RBC folate[2]	1006 (350)	1063 (536)	1107 (542)	0.1
	vitamin B6[2]	81.5 (36.8)	77.0 (35.6)	94.0 (48.3)	0.01
	vitamin B12[3]	288 (94)	289 (188)	361 (155)	0.07
	tHcy[4]	12.2 (3.2)	11.7 (4.1)	10.8 (4.4)	0.02
Semen	folate[2]	26.5 (11.6)	25.7 (16.4)	25.0 (15.5)	0.7
	vitamin B6[2]	30.5 (39.0)	30.0 (23.5)	28.5 (30.2)	0.3
	vitamin B12[3]	467 (608)	424 (450)	493 (339)	0.51
	tHcy[4]	4.1 (3.1)	4.4 (3.2)	3.7 (4.5)	0.02
Traditional Indian diet biomarker low intermediate high p5					
Blood	serum folate[2]	17.3 (7.7)	16.2 (6.7)	15.7 (9.1)	0.84
	RBC folate[2]	990 (554)	986 (382)	1110 (511)	0.04
	vitamin B6[2]	99.0 (48.0)	78.5 (31.0)	77.0 (36.5)	0.81
	vitamin B12[3]	329 (172)	293 (148)	282 (158)	0.41
	tHcy[4]	11.9 (4.7)	11.2 (3.1)	12.3 (3.7)	0.84
Semen	folate[2]	28.3 (14.8)	24.8 (10.5)	22.6 (15.8)	0.69
	vitamin B6[2]	33.0 (32.0)	29.0 (21.3)	29.0 (33.5)	0.42
	vitamin B12[3]	409 (406)	438 (400)	680 (440)	0.44
	tHcy[4]	3.7 (3.9)	4.0 (2.5)	4.7 (5.7)	0.62

[1]mean(sd), [2]nmol/L, [3]pmol/L [4]µmol/L. [5]Adjusted for age, BMI, smoking, vitamins, varicocele

Moreover, zinc acts as a cofactor for methionine synthase, an enzyme involved in the remethylation of homocysteine into methionine, thereby reducing levels of homocysteine. Regrettably, we were unable to gather any data regarding the levels of seminal poly-unsaturated fatty acids (PUFAs), a crucial element in determining if increased fish consumption would result in higher amounts of unsaturated fats that could potentially enhance the fluidity of the plasma membrane. However, it is important to note that a more fluid membrane also makes spermatozoa more susceptible to attack by reactive oxygen species (ROS), which could possibly explain why lifestyle factors that induce oxidative stress have been linked to a decline in human fertility over the past few decades. The fortuitous outcomes of zinc have been corroborated by a meticulously executed, randomized, and placebo-controlled intervention study carried out during the latter half of the 1990s. Within this study, the administration of zinc and/or folic acid to individuals experiencing subfertility demonstrated a remarkable surge in semen concentration, ranging from a noteworthy 18% to a staggering 74% increase. (Ebisch et al. 2006; Wong et al, 2002; Ebisch et al, 2006).

Received 08 November 2020; Accepted 15 December 2020.

Table 4:-Semen parameters according to the tertiles of the two major dietary patterns

Semen parameter	**low (n 40)**	**Health Conscious diet intermediate (n 44)**	**high (n 42)**	**p5**
DFI[2]	25.2 (21.1 - 29.2)	25.0 (20.2 - 29.8)	20.6 (17.5 - 23.6)	0.05
volume[3]	3.4 (2.9 - 3.8)	2.8 (2.4 - 3.2)	3.1 (2.6 - 3.5)	0.41
concentration[4]	46.2 (30.8 - 61.5)	39 (27.6 - 50.4)	48.2 (33.1 - 63.2)	0.89
motility[2]	35 (30 - 40)	33 (28 - 39)	37 (32 - 42)	0.06
morphology[2]	5 (4 - 6)	5 (4 - 6)	5 (4 - 6)	0.74
semen parameter	***low (n 44)***	***Traditional Indian diet intermediate (n 43)***	***high (n 39)***	***p5***
DFI[2]	23.5 (20.1 - 26.9)	25.0 (20.0 - 30.1)	22.0 (18.5 - 25.6)	0.53
volume[3]	3.1 (2.6 - 3.5)	3.1 (2.7 - 3.5)	3.0 (2.6 - 3.5)	0.89
concentration[4]	36.7 (26.8 - 46.6)	36.4 (23.1 - 49.8)	61.7 (44.5 - 78.9)	0.01
motility[2]	35 (31 - 39)	34 (29 - 40)	37 (31 - 43)	0.98
morphology[2]	5 (4 - 6)	5 (4 - 6)	6 (5 - 7)	0.34

Data are presented by [1]median with range, [2]percentages, [3]ml, [4]x106 cells/ml. [5]Adjusted for age, BMI, smoking, vitamin use and presence of a varicocele.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2210-2232

Received 08 November 2020; Accepted 15 December 2020.

Table 5:- Contribution of individual food groups to semen parameters

food group	*DFI*	*volume*	*Semen parameter concentration*	*motility*	*morphology*
alcohol	0.0 (-0.1; 0.1)	0.0 (-0.1; 0.1)	0.0 (-0.1; 0.2)	– 0.0 (–0.1; 0.1)	0.0 (-0.1; 0.1)
cereals	– 0.4 (-1.1; 0.4)	– 0.1 (-0.1; 0.1)	1.0 (-1.3; 3.2)	0.5 (0.2; 1.3)	0.0 (-0.2; 0.2)
butter	– 4.1 (-13; 4.8)	– 0.4 (-1.3; 0.4)	2.8 (–24; 30)	7.6 (-1.7; 17)	– 1.4 (-3.2; 0.4)
dairy	0.1 (-0.3; 0.4)	0.0 (0.0; 0.1)	0.5 (–0.6; 1.6)	– 0.1 (-0.5; 0.3)	0.0 (-0.1; 0.1)
eggs	– 0.3 (-0.9; 0.3)	0.1 (0.0; 0.1)[1]	– 0.2 (–2.1; 1.7)	– 0.1 (-0.7; 0.5)	0.0 (-0.1; 0.1)
fish	– 0.7 (-2.0; 0.6)	– 0.1 (-0.2; 0.1)	0.9 (–3.3; 5.1)	1.3 (0.0; 2.6)[1]	0.1 (-0.1; 0.4)
fruit	– 0.1 (-0.3; -0.0)[1]	0.0 (-0.1; 0.1)	– 0.1 (–0.5; 0.3)	0.2 (0.1; 0.3)[1]	0.0 (-0.1; 0.1)
legumes	– 0.5 (-1.2; 0.1)	– 0.0 (-0.1; 0.1)	0.2 (-1.7; 2.1)	0.1(-0.5; 0.8)	0.0 (-0.1; 0.1)
margarine	4.4 (-2.3; 11)	0.5 (-0.2; 1.2)	– 3.9 (-26; 18)	– 9.1 (-16; -1.5)[1]	0.2 (-1.3; 1.7)
mayonnaise	0.1 (-1.3; 1.6)	– 0.2 (-0.3; -0.1)[1]	– 0.1 (-4.3; 4.2)	– 0.8 (-2.3; 0.6)	– 0.1 (-0.4; 0.2)
meat	1.0 (-0.1; 2.0)	0.1 (-0.1; 0.2)	– 2.2 (-5.7; 1.4)	– 1.0 (-2.2; 0.2)	– 0.1 (-0.4; 0.1)
non-alc. drinks	0.0 (-0.1; 0.1)	0.0 (-0.1; 0.1)	– 0.3 (-0.5; -0.1)[1]	– 0.0 (-0.1; 0.1)	0.0 (-0.1; 0.1)
nuts	– 0.8 (-1.6; -0.1)[1]	– 0.1 (-0.2; 0.1)	– 1.0 (-3.4; 1.3)	0.2 (-0.6; 1.0)	0.1 (-0.1; 0.2)
refined grains	– 0.1 (-0.5; 0.2)	– 0.0 (-0.1; 0.1)	0.3 (-0.7; 1.3)	– 0.1 (-0.5; 0.2)	0.0 (-0.1; 0.1)
potatoes	– 0.1 (-0.2; 0.1)	0.0 (-0.1; 0.1)	0.8 (0.2; 1.4)[1]	0.1 (-0.1; 0.3)	0.0 (-0.1; 0.1)
sauces	– 0.8 (-1.8; 1.6)	– 0.1 (-0.3; 0.1)	0.2 (-5.3; 5.7)	– 0.8 (-2.6; 1.0)	– 0.0 (-0.4; 0.3)
snacks	0.1 (-0.4; 0.5)	0.0 (-0.1; 0.1)	– 0.6 (-2.1; 1.0)	0.2 (-0.3; 0.7)	– 0.1 (-0.2; 0.0)
soup	0.0 (-0.1; 0.1)	0.0 (-0.1; 0.1)	– 0.2 (-0.4; -0.0)[1]	– 0.0 (-0.1; 0.1)	0.0 (-0.1; 0.1)
sweets	0.4 (-0.4; 1.3)	– 0.0 (-.01; 0.1)	0.3 (-2.5; 3.1)	– 0.1 (-0.9; 0.8)	– 0.1 (-0.2; 0.1)
vegetable oil	– 1.1 (-5.2; 3.0)	– 0.3 (-0.7; 0.2)	– 3.3 (-17; 10)	1.1 (-3.4; 5.6)	– 0.2 (-1.1; 0.7)
vegetables	– 0.3 (-0.5; -0.0)[1]	– 0.0 (-0.1; 0.1)	0.8 (0.0; 1.6)[1]	0.3 (0.1; 0.6)[1]	0.0 (-0.1; 0.1)
whole grains	– 0.2 (-0.3; 0.0)	0.0 (-0.1; 0.1)	0.4 (-0.2; 1.0)	0.3 (-0.0; 0.4)	0.0 (-0.1; 0.1)

Data are presented by mean with 95% CI. [1]p ≤0.05, after adjustments for age, BMI, smoking and vitamin use.

In a smaller group of men, as part of the FOLFO study, we examined the biomarkers of homocysteine metabolism in blood and seminal plasma to explore their connection to semen parameters. We found that seminal plasma vitamin B12 was positively associated with sperm concentration (Boxmeer et al, 2007). In our current analysis, we discovered a significant correlation between the Traditional Indian dietary pattern and semen concentrations. In fact, the highest tertile of this diet saw an increase in semen concentration from $36.7x10^6$ to $61.7x10^6$ cells/ml (p 0.01). We also observed a positive, though not statistically significant, association between the Traditional Indian dietary pattern and seminal vitamin B12 concentrations. This is likely due to the high meat intake in this diet, which aligns with the previously observed link between seminal vitamin B12 and sperm concentration. A recent study by Mendiola et al. (2008) suggested that meat intake has a negative impact on semen quality, attributing it to the presence of natural and synthetic estrogens in meat. However, their study was limited in size (n = 61) and only focused on individual food items,

Received 08 November 2020; Accepted 15 December 2020.

without considering dosage effects or portion sizes. It is possible that the positive effect of the meat-rich Traditional Indian diet on semen quality is a result of the relatively lower meat consumption in our country, or perhaps due to lower exposure of Indian cattle to hormones and endocrine disruptors. It is important to note that there are some concerns about the study design. While we adjusted for factors such as age, energy intake, BMI, smoking, and vitamin supplement use, there may be other lifestyle factors that could confound the identified dietary patterns. Factors such as exercise, psychological stress, and exposure to environmental pollutants could potentially influence the results (Homan et al, 2007). To mitigate the possibility of multiple testing, we used principal component analysis to summarize overall nutrient intake into two dietary patterns. By focusing on just two variables of exposure and considering all nutrients, we have successfully avoided the issue of selective testing. These two variables were found to have connections to biomarkers of homocysteine metabolism and semen parameters, ensuring that our findings were not merely a result of chance. It is important to note that all methods of dietary assessment are prone to error. We used a Food Frequency Questionnaire (FFQ) to evaluate nutritional intake. However, it is common for individuals to underestimate their energy intake when self-reporting their diet (Black et al, 1991). To address this, we assessed the presence of underreporting by estimating the Physical Activity Level (PAL). The average PAL for all participants was determined to be 1.28, indicating an underestimation of energy intake in both fertile and sub-fertile men according to the Goldberg standard (PAL $\leq$1.35) (Goldberg et al, 1991). We believe it is unlikely that underestimation has distorted the associations between semen parameters and energy. While we acknowledge that these findings may not be applicable to other populations with different dietary patterns and food production methods, they still provide valuable insights for a significant portion of the global population and their lifestyles. The study's potency lies within its forward-thinking approach and the considerable number of participants, totaling 172 men. This allowed us to generate dependable dietary patterns that could be compared to similar investigations carried out among the public at large (Van et al, 2003).

General Conclusion

The correlation between the Health Conscious and Traditional Indian dietary patterns and semen quality has aroused intrigue. However, a deeper exploration is imperative to unravel the causal link between these associations and their profound impact on human reproduction.

Bibliography

1. Abbasi, A., Prasad, A., Rabbani, P., (1979). Experimental zinc deficiency in man: effect on spermatogenesis. Trans Assoc Am Physicians;92:292–302.
2. Afeiche, M., Williams, P.L., Mendiola, J., Gaskins, A., Jorgensen, N., Swan, S.H., Chavarro, J.E., (2013). Dairy food intake in relation to semen quality and reproductive hormone levels among physically active young men. Hum Reprod;28:2265–2275.
3. Aitken, R.J., Gibb, Z., Baker, M.A., Drevet, J., Gharagozloo, P., (2016). Causes and consequences of oxidative stress in spermatozoa. Reprod Fertil Dev;28:1–10.
4. Aksoy, Y., Aksoy, H., Altinkaynak, K., Aydin, H.R., Ozkan, A., (2006). Sperm fatty acid composition in subfertile men. Prostaglandins Leukot Essent Fatty Acids;75:75–79.
5. Amarnath, R,, Jesse, N.M., Jacob, R., (2016). The role of estrogen modulators in male hypogonadism and infertility. Rev Urol;18:66–72.

6. Anderson, J.E., Farr, S.L., Jamieson, D.J., Warner, L., Macaluso, M., (2009). Infertility services reported by men in the United States: national survey data. Fertil Steril;91: 2466–2470.
7. Atkinson, F., Foster, K., Brand, J.C., (2008). International tables of glycemic index and glycemic load values: Diabetes Care 2008;31:2281–2283.
8. Black, A.E., Goldberg, G.R., Jebb, S.A., Livingstone, M.B., Cole, T.J., Prentice, A.M., (1991). Critical evaluation of energy intake data using fundamental principles of energy physiology: 2. Evaluating the results of published surveys. Eur J Clin Nutr;45:583-99.
9. Boivin, J., Bunting, L., Collins, J.A., Nygren, K.G., (2007). International estimates of infertility prevalence and treatment-seeking : potential need and demand for infertility medical care. Hum Reprod;22:1506–1512.
10. Bonjour, J.P., Benoit, V., Rousseau, B., Souberbielle, J.C., (2012). Consumption of vitamin Dand calcium-fortified soft white cheese lowers the biochemical marker of bone resorption TRAP 5b in postmenopausal women at moderate risk of osteoporosis fracture. J Nutr;142:698–703.
11. Boxmeer, J.C., Smit, M., Utomo, E., (2008). Low folate in seminal plasma is associated with increased sperm DNA damage. Fertil Steril;92:548-56.
12. Boxmeer, J.C., Smit, M., Weber, R.F., (2007). Seminal plasma cobalamin significantly correlates with sperm concentration in men undergoing IVF or ICSI procedures. J Androl;28:521-527.
13. Collins, G.G., Rossi, B.V., (2015). The impact of lifestyle modifications, diet, and vitamin supplementation on natural fertility. Fertil Res Pract;1:11.
14. Comhaire, F.H., Christophe, A.B., Zalata, A.A., Dhooge, W.S., Mahmoud, A.M., Depuydt, C.E., (2000). The effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in subfertile men. Prostaglandins Leukot Essent Fatty Acids;63:159-65.
15. Diamanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., (2009). Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev;30:293–342.
16. Ding, G.L., Liu, Y., Liu, M.E., Pan, J.X., Guo, M.X., Sheng, J.Z., Huang, H.F., (2015). The effects of diabetes on male fertility and epigenetic regulation during spermatogenesis. Asian J Androl;17:948–953.
17. Donders, M., van der Heijden, L., Hulshof, K.F., (2003). Informatorium voor voeding en dietetiek. Wageningen: Human Nutrition of TNO and Wageningen University.
18. Dong, J.Y., Zhang, L., Zhang, Y.H., Qin, L.Q., (2011). Dietary glycaemic index and glycaemic load in relation to the risk of type 2 diabetes: a meta-analysis of prospective cohort studies. Br J Nutr;106:1649–1654.
19. Ebisch, I.M., Peters, W.H., Thomas, C.M., Wetzels, A.M., Peer, P.G., Steegers, R.P., (2006). Homocysteine, glutathione and related thiols affect fertility parameters in the (sub)fertile couple. Hum Reprod;21:1725-33.
20. Ebisch, I.M., Pierik, F.H., Thomas, C.M., Steegers, R.P., (2006). Does folic acid and zinc sulphate intervention affect endocrine parameters and sperm characteristics in men? Int J Androl;29:339-45.
21. Ebisch, I.M., Thomas, C.M., Peters, W.H., Braat, D.D., Steegers, R.P., (2007). The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. Hum Reprod Update;13:163-74.

Received 08 November 2020; Accepted 15 December 2020.

22. Esmaeili, V., Shahverdi, A.H., Moghadasian, M.H., Alizadeh, A.R., (2015). Dietary fatty acids affect semen quality: a review. Andrology;3:450–461.
23. Evers, J.L.H., (2002). Female subfertility. Lancet;360:151–159.
24. Favier, A.E., (1992). The role of zinc in reproduction. Hormonal mechanisms. Biol Trace Elem Res;32:363-82.
25. Feunekes, G.I., Van Staveren, W.A., De Vries, J.H., Burema, J., Hautvast, J.G., (1993). Relative and biomarker- based validity of a food-frequency questionnaire estimating intake of fats and cholesterol. Am J Clin Nutr;58:489-96.
26. Ganmaa, D., Qin, L., Wang, P., (2004). A two generation reproduction study to assess the effects of cows' milk on reproductive development in male and female rats. Fertil Steril;82:1106–1114.
27. Ghanem, H., Shaeer, O., El-Segini, A., (2010). Combination clomiphene citrate and antioxidant therapy for idiopathic male infertility: a randomized controlled trial. Fertil Steril;93:2232–2235.
28. Giahi, L., Mohammadmoradi, S., Javidan, A., Sadeghi, M.R., (2016). Nutritional modifications in male infertility: a systematic review covering 2 decades. Nutr Rev;74: 118–130.
29. Goldberg, G.R., Black, A.E., Jebb, S.A., (1991). Critical evaluation of energy intake data using funda- mental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. Eur J Clin Nutr;45:569-81.
30. Goldin, B., Adlercreutz, H., Gorbach, S., Warram, J., Dwyer, J., Swenson, L., Woods, M., (1982). Estrogen excretion patterns and plasma levels in vegetarian and omnivorous women. N Engl J Med;307:1542–1547.
31. Gulaya, N.M., Margitich, V.M., Govseeva, N.M., Klimashevsky, V.M., Gorpynchenko, I.I., Boyko, M.I., (2001). Phospholipid composition of human sperm and seminal plasma in relation to sperm fertility. Arch Androl;46:169–175.
32. Hansen, M.L., Thulstrup, A.M., Bonde, J.P., Olsen, J., Håkonsen, L.B., Ramlau-Hansen, C.H., (2012). Does last week's alcohol intake affect semen quality or reproductive hormones? A cross-sectional study among healthy young Danish men. Reprod Toxicol; 34:457–462.
33. Hassan, M.A.M., Killick, S.R., (2004). Negative lifestyle is associated with a significant reduction in fecundity. Fertil Steril;81:384–392.
34. Henricks, D.M., Gray, S.L., Owenby, J.J., Lackey, B.R., (2001). Residues from anabolic preparations after good veterinary practice. Apmis;109:273-83.
35. Homan, G.F., Davies, M., Norman, R., (2007). The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatments: a review. Hum Reprod Update;13:209-23.
36. Hu, Y., Block, G., Norkus, E.P., Morrow, J.D., Dietrich, M., Hudes, M., (2006). Relations of glycemic index and glycemic load with plasma oxidative. Am J Clin Nutr;84:70–76.
37. Huang, P., Zhou, Z., Shi, F., Shao, G., Wang, R., Wang, J., Wang, K., Ding, W., (2016). Effects of the IGF-1/PTEN/Akt/FoxO signaling pathway on male reproduction in rats subjected to water immersion and restraint stress. Mol Med Rep;5116–5124.
38. Imamura, F., O'Connor, L., Ye, Z., Mursu, J., Hayashino, Y., Bhupathiraju, S.N., Forouhi, N.G., (2015). Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: systematic review, metaanalysis, and estimation of population attributable fraction. BMJ;351:h3576.

39. Jungwirth, A., Giwercman, A., Tournaye, H., Diemer, T., Kopa, Z., Dohle, G., Krausz, C., (2012). European association of urology guidelines on male infertility: Eur Urol 2012;62:324–332.
40. Jurewicz, J., Radwan, M., Sobala, W., Ligocka, D., Radwan, P., Bochenek, M., Hawula, W., Jakubowski, L., Hanke, W., (2013). Human urinary phthalate metabolites level and main semen parameters, sperm chromatin structure, sperm aneuploidy and reproductive hormones. Reprod Toxicol;42:232–241.
41. Kaiser, H.F., (1958). The Varimax Criterion for Analytic Rotation in Factor Analysis. Psychometrika;23:187-200.
42. Kristo, A.S., Matthan, N.R., Lichtenstein, A.H., (2013). Effect of diets differing in glycemic index and glycemic load on cardiovascular risk factors: review of randomized controlled-feeding trials. Nutrients;5:1071–1080.
43. La Vignera, S., Condorelli, R.A., Balercia, G., Vicari, E., Calogero, A.E., (2013). Does alcohol have any effect on male reproductive function? A review of literature. Asian J Androl;15:221–225.
44. Malik, V.S., Pan, A., Willett, W.C., Hu, F.B., (2013). Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. Am J Clin Nutr;98:1084–1102.
45. Malik, V.S., Popkin, B.M., Bray, G.A., Despres, J.P., Willett, W.C., Hu, F.B., (2010). Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. Diabetes Care;33:2477–2481.
46. Maneesh, M., Dutta, S., Chakrabarti, A., (2006). Alcohol abuse-duration dependent decrease in plasma testosterone and antioxidants in males. Indian J Physiol Pharmacol; 50:291–296.
47. Mendiola, J., Jorgensen, N., Mínguez, L., Sarabia, L., Lopez, J.J., Vivero, G., Ruiz, K.J., Fernandez, M.F., Olea, N., Swan, S.H., (2013). Sperm counts may have declined in young university students in Southern Spain. Andrology;1:408–413.
48. Mendiola, J., Torres, A.M., Moreno, J.M., (2008). Food intake and its relationship with semen quality: a case-control study. Fertil Steril;91:812-8.
49. Merzenich, H., Zeeb, H., Blettner, M., (2010). Decreasing sperm quality: a global problem? BMC Public Health;10:1–5.
50. Miki, K., (2007). Energy metabolism and sperm function. Soc Reprod Fertil Suppl;65: 309–325.
51. Mitchell, M., Bakos, H.W., Lane, M., (2011). Paternal diet induced obesity impairs embryo development and implantation in the mouse. Fertil Steril;95:1349–1353.
52. Molloy, A.M., (2012). Genetic aspects of folate metabolism. In: Stanger O (ed). Water Soluble Vitamins; Vol 18. Netherlands: Springer;105–130.
53. Morgan, D.H., Ghribi, O., Hui, L., Geiger, J.D., Chen, X., (2014). Cholesterol enriched diet disrupts the blood-testis barrier in rabbits. Am J Physiol Endocrinol Metab;307: 1125–1130.
54. Muraki, I., Rimm, E.B., Willett, W.C., Manson, J.E., Hu, F.B., Sun, Q., (2016). Potato consumption and risk of type 2 diabetes: results from three prospective cohort studies. Diabetes Care;39:376–384.
55. Ng, S.F., Lin, R.C.Y., Laybutt, D.R., Barres, R., Owens, J.A., Morris, M.J., (2010). Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. Nature;467: 963–966.
56. Palmer, N.O., Bakos, H.W., Fullston, T., Lane, M., (2012). Impact of obesity on male fertility, sperm function and molecular composition. Spermatogenesis;2:253–263.

Received 08 November 2020; Accepted 15 December 2020.

57. Park, K., Gross, M., Lee, D.H., Holvoet, P., Himes, J.H., Shikany, J., Jacobs, J.D.R., (2009). Oxidative stress and insulin resistance: the coronary artery risk development in young adults study. Diabetes Care;32:1302–1307.
58. Pfeiffer, C.M., Huff, D.L., Gunter, E.W., (1999). Rapid and accurate HPLC assay for plasma total homocysteine and cysteine in a clinical laboratory setting. Clin Chem;45:290-2.
59. Ramlau, C.H., Thulstrup, A.M., Bonde, J.P., Olsen, J., Bech, B.H., (2008). Semen quality according to prenatal coffee and present caffeine exposure: two decades of follow-up of a pregnancy cohort. Hum Reprod;23:2799–2805.
60. Rato, L., Alves, M.G., Cavaco, J.E., Oliveira, P.F., (2014). High energy diets: a threat for male fertility? Obes Rev;15:996–1007.
61. Ricci, E., Al, Beitawi, S., Cipriani, S., Candiani, M., Chiaffarino, F., Vigano, P., Noli, S., Parazzini, F., (2016). Semen quality and alcohol intake: a systematic review and metaanalysis. Reprod Biomed Online;34:38–47.
62. Ross, C., Morriss, A., Khairy, M., Khalaf, Y., Braude, P., Coomarasamy, A., El, T., (2010). A systematic review of the effect of oral antioxidants on male infertility. Reprod Biomed Online;20:711–723.
63. Rozati, R., Reddy, P.P., Reddanna, P., Mujtaba, R., (2002). Role of environmental estrogens in the deterioration of male factor fertility. Fertil Steril;78:1187–1194.
64. Ruff, J.S., Suchy, A.K., Hugentobler, S.A., Sosa, M.M., Schwartz, B.L., Morrison, L.C., Gieng, S.H., Shigenaga, M.K., Potts, W.K., (2013). Human relevant levels of added sugar consumption increase female mortality and lower male fitness in mice. Nat Commun;4:2245.
65. Safarinejad, M.R., (2011). Effect of omega-3 polyunsaturated fatty acid supplementation on semen profile and enzymatic anti-oxidant capacity of seminal plasma in infertile men with idiopathic oligoasthenoteratospermia: a double-blind, placebocontrolled, randomised study. Andrologia;43:38–47.
66. Santti, R., Makela, S., Strauss, L., Korkman, J., Kostian, M., (1998). Phytoestrogens: potential endocrine disruptors in males. Toxicol Ind Health;14:223–237.
67. Schoeller, E.L., Albanna, G., Frolova, A.I., Moley, K.H., (2012). Insulin rescues impaired spermatogenesis via the hypothalamic-pituitary- gonadal axis in Akita diabetic mice and restores male fertility. Diabetes;61:1869–1878.
68. Schofield, W.N., (1985). Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr;39:5-41.
69. Schrijver, J., Speek, A.J., Schreurs, W.H., (1981). Semi-automated fluorometric determination of pyridoxal- 5'-phosphate (vitamin B6) in whole blood by high-performance liquid chromatography (HPLC). Int J Vitam Nutr Res;51:216-22.
70. Showell, M., Mackenzie, R., Brown, J., Yazdani, A., Stankiewicz, M., Hart, R., (2014). Antioxidants for male subfertility. Cochrane Database Syst Rev Antioxid;1:1–188.
71. Slimani, N., Fahey, M., Welch, A.A., (2002). Diversity of dietary patterns observed in the European Prospective Investigation into Cancer and Nutrition (EPIC) project. Public Health Nutr;5:1311-28.
72. Smit, M., Dohle, G.R., Hop, W.C., Wildhagen, M.F., Weber, R.F., Romijn, J.C., (2007). Clinical correlates of the biological variation of sperm DNA fragmentation in infertile men attending an andrology outpatient clinic. Int J Androl;30:48-55.
73. Stanhope, K.L., Schwarz, J.M., Keim, N.L., Griffen, S.C., Bremer, A.A., Graham, J.L., Hatcher, B., Cox, C.L., Dyachenko, A., Zhang, W., (2009). Consuming fructose sweetened, not

glucose-sweetened, beverages increase visceral adiposity and lipids and decrease insulin sensitivity in overweight/obese men. J Clin Invest;1334:1322–1334.

74. Swan, S.H., Liu, F., Overstreet, J.W., Brazil, C., Skakkebaek, N.E., (2007). Semen quality of fertile US males in relation to their mothers' beef consumption during pregnancy. Hum Reprod;22:1497–1502.
75. The World Bank Group, (2005).
76. Van, R.M., Grievink, L., Ocke, M.C., Feskens, E.J., (2003). Patterns of food consumption and risk factors for cardiovascular disease in the general Dutch population. Am J Clin Nutr;77:1156-63.
77. Veaute, C., Andreoli, M.F., Racca, A., Bailat, A., Scalerandi, M.V., Bernal, C., Borel, I.M., (2007). Effects of isomeric fatty acids on reproductive parameters in mice. Am J Reprod Immunol;58:487–496.
78. Verkleij, A.C., de Vries, J.H., Ursem, N.T., Hop, W.C., (2006). Dietary intake of B-vitamins in mothers born a child with a congenital heart defect. Eur J Nutr;45:478-86.
79. Vitvitsky, V., Prudova, A., Stabler, S., Dayal, S., Lentz, S.R., Banerjee, R., (2007). Testosterone regulation of renal cystathionine beta-synthase: implications for sex-dependent differences in plasma homocys- teine levels. Am J Physiol Renal Physiol;293:F594-600.
80. Vujkovic, M., Ocke, M.C., van der Spek, P.J., Yazdanpanah, N., Steegers, E.A., Steegers, R.P., (2007). Maternal Western dietary patterns and the risk of developing a cleft lip with or without a cleft palate. Obstet Gynecol;110:378-84.
81. Wallock, L.M., Tamura, T., Mayr, C.A., Johnston, K.E., Ames, B.N., Jacob, R.A., (2001). Low seminal plasma folate concentrations are associated with low sperm density and count in male smokers and non- smokers. Fertil Steril;75:252-9.
82. Wathes, D.C., Abayasekara, D., Aitken, R.J., (2007). Polyunsaturated fatty acids in male and female reproduction. Biol Reprod;77:190–201.
83. Willett, W.C., Howe, G.R., Kushi, L.H., (1997). Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr;65:1220S-1228S; 1229S-1231S.
84. Williams, A., Ford, W., (2001). The role of glucose in supporting motility and capacitation in human spermatozoa. J Androl;22:680–695.
85. Wong, W., Merkus, H., Thomas, C., Menkveld, R., Zielhuis, G., Steegers, R., (2002). Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial. Fertil Steril;77:491–498.
86. Wong, W.Y., Thomas, C.M., Merkus, J.M., Zielhuis, G.A., (2000). Steegers-Theunissen RP. Male factor subfertil- ity: possible causes and the impact of nutritional factors. Fertil Steril;73:435-42.
87. World Health Organization, (1999). WHO laboratory manual for the examination of human semen and sperm–cervical mucus interaction. 4 ed. Cambridge: Cambridge University Press.
88. Young, S.S., Eskenazi, B., Marchetti, F.M., Block, G., Wyrobek, A.J., (2008). The association of folate, zinc and antioxidant intake with sperm aneuploidy in healthy non-smoking men. Hum Reprod; 23:1014-22.