

Efficacy of antioxidant supplementation on sperm quality in men with oligoasthenoteratozoospermia

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Abstract

Introduction

Evidence of a positive relationship between the intake of antioxidant supplements and the improvement of sperm parameters has been reported mainly in European and American populations and very little in Asian populations. Hence, we aimed to investigate the association between antioxidant supplement intake (i.e., Profertil®) and sperm parameters in Malaysian men with Oligoasthenoteratozoospermia (OAT).

Materials and methods: This is a single-centre retrospective study that investigated a total of 195 men with OAT whose female partners underwent ART treatment between 2016 and 2021. They were divided into 2 groups; a treatment group where male patients received two daily capsules of the antioxidant supplement (Profertil®) for 3 months (n = 92) and a control group where no micronutrient was given to the male partner (n = 103). Sperm parameters, including semen volume, sperm concentration, progressive motility, total motility, and morphology, were analyzed before and after treatment. A Kruskal-Wallis test was performed to compare the median levels of the sperm parameters. A Chi-square test was performed to compare the frequencies of normal morphology between the studied groups.

Results: Demographic and baseline characteristics showed no significant differences except for the mean age of the husband and the etiology of infertility ($p < 0.05$). When compared to their baseline sperm quality, men receiving Profertil® had highly significant increases in median sperm concentration ($p < 0.0001$), motility ($p < 0.0001$), progressive motility ($p < 0.0001$), total sperm count ($p < 0.0001$), total sperm motility ($p = 0.01$) and total progressive motility ($p < 0.0001$). Moreover, sperm abnormality of a total of 24 (26.1%) men improved after receiving Profertil® ($p = 0.013$).

Conclusion: Our data showed that antioxidants (Profertil®) have a positive effect on semen parameters in men with OAT.

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Introduction

Mascarenhas et al. (2012) found an estimated 48.5 million couples worldwide were infertile. Over the past few decades, a steady decline in semen quality among men has been observed across the world since its first publication by

Carlsen et al. (1992, cited in: Levine et al., 2017). Winters and Walsh (2014) claimed that the male factor contributes to approximately half of subfertility cases, either alone or in combination with female factors, and it is becoming a public

health issue for several reasons. The economic and societal burden of male infertility is high and increasing, leading to a decreasing population (Winters and Walsh, 2014; Hauser et al., 2015; Skakkebaek et al., 2016); an increase in all-cause mortality and morbidity (Jensen et al., 2009; Eisenberg et al., 2016); and a reduction in fertility and birth rate (Skakkebaek et al., 2016; Borges et al., 2016).

Intracytoplasmic sperm injection (ICSI) is a common mechanical technique used to overcome male infertility problems whereby sperm is selected to penetrate the oocyte easily through the zona pellucida (ZP). ICSI generally achieves a fertilization rate of about 70 to 80% (Cardona et al., 2020; Palermo et al., 2017; Neri et al., 2014; Vanden Meerschaut et al., 2014), but total fertilization failure (TF) still occurs in 1 to 3% of ICSI cycles (Bhattacharya et al., 2013). Cancellation of embryo transfer has been reduced where ICSI is used. This suggests that ICSI increases the chances of fertilization but is not associated with improved reproductive outcomes compared to the in vitro fertilization (IVF) method in couples with male infertility factors (Boulet et al., 2015). Alasi et al. (2018) revealed that fertilization rates were higher in the unexplained infertility group (95%) than in the severe (88%) and mild male infertility groups (90%). However, the number of good-quality embryos and biochemical pregnancy rates were higher in the severe and mild male infertility groups. Still, there was no significant difference in the clinical pregnancy rates between all groups.

Oxidative stress is induced by reactive oxygen species (ROS), or free radicals, when the production of reactive oxygen species (ROS) exceeds the body's natural antioxidant defenses, leading to oxidative stress (Pizzino et al., 2017). A spermatozoon requires a small amount of reactive oxygen (ROS) to provide beneficial functional effects, especially in sperm capacitation, hyperactivation, sperm-oocyte fusion, regulation of sperm maturation, and enhancement of cellular signaling pathways (Agarwal et al., 2014). Lipid-peroxidation-induced damage and pathological changes in sperm deoxyribonucleic acid (DNA) will occur when excessive ROS is produced (Aitken, 2017; Moazamian et al., 2015).

Sperm exposed to high levels of ROS have a reduction in normal morphology, viability, and motility as measured by conventional assessment and computer-assisted sperm motility analysis (de Castro et al., 2016; Shi et al., 2012). Also, high ROS concentration in infertile men has been associated with DNA fragmentation and poor chromatin packing, thus increasing miscarriage or pregnancy loss and the potential for birth defects (Agarwal et al., 2012). Besides that, excess ROS causes damage to both mitochondrial and nuclear DNA of sperm at an amino acid, breaking and attacking the phosphodiester backbones on which it holds the nucleotides of DNA or RNA polymers together and producing base-free sites, point mutations, polymorphisms, deletions, translocations, strand breaks, chromatin cross-links, frame shifts, and even rearranging chromosomes that can result in abnormal chromosomes with more than one centromere (Lourenco et al., 2019; Bisht et al., 2017).

Most studies report that infertile men have been identified as having lower levels of antioxidants in their semen compared to fertile controls, thereby exposing these men to an increased risk of ROS damage to their sperm. Thus, antioxidants are required to help maintain ROS equilibrium in the redox potential for optimal sperm function. Various efforts are made for male infertility problems, such as the production of drugs or supplements or treatment by researchers. Thus, interest in the use of antioxidants to treat male infertility is growing. A systematic review by Majzoub and Agarwal (2017) demonstrated that the use of antioxidant supplementation in infertile males might significantly impact sperm quality and reproduction. In recent years, many oral micronutrient products have been available on the market due to awareness of the effect of oxidative stress on male infertility and the role of these supplements in improving semen properties in infertile men. Antioxidant supplements are widely available, easy to consume, inexpensive and also have a good safety profile compared to other treatments such as empirical hormonal therapies including aromatase inhibitors and selective estrogen receptor modulators (Vessey et al., 2020; Showell et al., 2015; Jung and Seo, 2014). Most studies show a positive relationship between antioxidants and improved male infertility (Son

and Gadallah, 2018; Majzoub and Agarwal, 2018; Lipovac et al., 2016; Showell et al., 2015; Lipovac et al., 2014; Showell et al., 2013). However, all the evidence was reported mainly in European and American populations and very little in Asian populations, especially Malaysia. As a result, we sought to investigate the relationship between antioxidant supplement consumption (i.e., Profertil®) and sperm parameters in Malaysian men suffering from oligoasthenoteratozoospermia (OAT).

Materials and methods

Patient recruitment

This is a retrospective study recruited from all infertile male partners whose female partners were undergoing Intracytoplasmic Sperm Injection (ICSI) at the Reproductive Medicine Unit, Hospital Tunku Azizah (Hospital Wanita Dan Kanak-Kanak Kuala Lumpur). This study was extracted from 1 January 2016–30 September 2021. They were divided into two groups; the treatment group, whereby the male partner took two daily capsules of the antioxidant supplement (Profertil®) (LenusPharma, Vienna, Austria) for 3 months as recommended by a clinician, and the control group, whereby they did not consume any micronutrient treatment. In the treatment (i.e., Profertil®), male partners repeated the semen analysis after 3 months of consuming supplements.

Inclusion and exclusion criteria

This study included all infertile male partners with at least one year of sub-/infertility and at least one prior age range of 20 to 50 years, as well as one recent abnormal sperm analysis with OAT, which is defined as low sperm count (15 million), low motility (32%), and low normal morphology of less than 4% (Cooper et al., 2010; WHO, 2010). As for exclusion criteria, all infertile male partners with azoospermia, aspermia, varicocele, and recent urogenital infections are excluded. We also excluded diabetes, inflammatory disease, chronic kidney, and renal disease patients who took hormone therapy or antioxidant supplements.

Ethical approval

This study was conducted in compliance with ethical principles outlined in the MREC, whose investigations were approved by the local ethical

committee MREC and the Clinical Research Center (CRC) at Hospital Tunku Azizah. All information about subjects was enrolled in the study, including a justification for their inclusion criteria.

Semen analysis

Following 2–5 days of abstinence, semen samples were obtained by masturbation in a private room provided. The samples were stored at 20–30°C for about 30 minutes until liquefaction was complete. Sperm parameters were analyzed according to World Health Organization (WHO) 2010 guidelines, including sperm count, motility, morphology, and viability (Cooper et al., 2010). A second semen analysis was performed after 3 months of consuming antioxidant supplement intake (Profertil®) as suggested by a clinician.

Sperm count

Sperm counts were carried out using the Makler® Counting Chamber (Makler A, 2003; Makler A, 1980). The counting was done in the ruled squares on the grid. If the number of sperm was substantial, their number in a strip of 10 squares was counted. This number represents their concentration in millions per mL. The Makler® Counting Chamber was heated at 37°C before using it for around 10 minutes in the incubator or on the heating stage. The semen sample was mixed well to avoid the formation of bubbles. One drop (5–10 µl) was dropped with the aid of a wooden rod or pipette from the semen sample onto the flat disc made of optical flat glass on which the sample is placed. The cover glass was placed on the four pins immediately. Once the cover glass is in place, avoid touching, lifting, and covering it again, as this may change the uniform spread of sperm within the chamber. The chamber was lifted by its handles and placed on the stage of the microscope, and the chamber grip was used to fit it properly. With 200x magnification, was used to observe and count the number of sperm with a row of 10 squares = sperm density x 10⁶/ml. The count was made from 2 or 3 other drops of the specimen to determine the average and increase the reliability of the count determination. The spermatozoa with head and tail were counted. In the case of oligospermic specimen, it is suggested to count sperm in the entire grid are and divided by 10 due to have 10 columns. Example, if < 5 sperms per row of 10

squares are seen, then the entire grid were counted and divided by 10. The result was the concentration in millions per mL with a row of 100 squares = sperm density /10 columns x 106/ml. The space bounded in a row of 10 squares is exactly one millionth of mL. Therefore, the number of sperm heads in 10 squares were indicates their concentration in million/ mL.

Sperm motility

The sperm motility was counted at the same time as the sperm count using the Makler® Counting Chamber (Makler A, 2003; Makler A, 1980). One drop (5-10µl) was dropped with the aid of a wooden rod or pipette from the semen sample onto the flat disc made of optical flat glass on which the sample is placed. The cover glass was placed on the four pins immediately. The motile and immotile sperm were observed at x200 magnification. The sperm motility was graded based on criteria set by WHO (2010), which consists of progressive motility, non-progressive motility, and immotile sperm (Coa et al., 2011). The percentage of motile sperm was recorded. Sperm motility was expressed as a percentage of motile sperm with both forward and non-progressive counts of total sperm.

Sperm anatomy

Sperm morphology was carried out by computer-aided semen analysis (CASA) after the smeared slide was stained with the Diff-Quick Staining technique (Baxter, Australia). Firstly, slide preparation for sperm morphology (the feathering method (WHO, 2010) was prepared by dropping 3-5 µl of semen sample onto the slide. Then, the slide was labelled with the patient's name, ID number, and date. A drop (3 to 5 µl) of semen was applied near the frosted end of the slide. The drop of semen along the surface of the slide backward and pushed across the length of the slide was pulled with a second slide, and then the slide was left to air-dry. In the case of severe oligospermia (concentration $\leq 5 \times 10^6$), the semen sample was spun, and the supernatant removed until 0.1-0.3 ml. The pellet was mixed and 3-5 µl was taken to prepare the slide.

After that, in the Rapid Staining procedure using the Diff-Quick staining set (WHO, 2010), the slide was dipped in Ethanol for 10 seconds, then dipped in Diff-Quick 1 for 20 seconds, and

lastly in Diff-Quick 2 for 20 seconds. To remove excess stain, the dipped slide was cleaned with running water. The excess solution was drained at each step by placing the stained slide vertically on absorbent paper. The stained slide will be examined with bright field optics at 1000 magnification with oil immersion to gain more focus. Use Computer Aided Sperm Analysis (CASA) software for morphology evaluation. Approximately 200 spermatozoa were assessed and the value of normal sperm was calculated in percentage. If the sperm concentration was low ($< 2 \times 10^6$ per mL), samples could be concentrated by centrifugation (centrifugation may affect sperm morphology).

The viability of sperm

1 % Eosin Y was aliquoted and mixed thoroughly with 2 drops of reagent, 1 % Eosin Y in an Eppendorf tube. After 30 seconds, 3 drops of 5% Nigrosin were added and mixed thoroughly. Within 30 seconds of adding 5% Nigrosin reagent, 20 ul of the semen-stain mixture was placed on a microscope slide and immediately placed a coverslip on top before being observed under x100 oil immersion with a light microscope. The dead sperm showed a pink coloration of the head, whereas the viable sperm showed a whitish or colorless head. Approximately 200 sperm were observed for dead and live cells, and the percentage of dead and live cells was recorded.

Sample Disposal

All utilized semen specimens were discarded into the yellow biological hazards bag.

Statistical Analysis

Statistical analysis was performed using the SPSS software package (Version 10.0 for Windows, SPSS Inc., Chicago, Illinois, USA). The information was presented as a mean (minimum and maximum values), median, interquartile range, and percentage. Descriptive data was expressed as mean standard deviation (SD) unless otherwise stated. The Kolmogorov-Smirnov test was used to determine the normality of data distribution. According to this, differences between groups were analyzed with the Mann Whitney test (continuous nonparametric data), whereas changes within each studied group were evaluated with the Wilcoxon rank test. A $p < 0.05$ level of

significance was considered statistically significant.

Results

In total, 195 (of 200) sub-/infertile men whose female partners were undergoing Intracytoplasmic Sperm Injection (ICSI) treatment at the Reproductive Medicine Unit, Hospital Tunku Azizah (Hospital Wanita Dan Kanak-Kanak Kuala Lumpur) were recruited for the study. These patients had at least 1 year of sub-/infertility and at least one prior and one recent abnormal semen analysis. These males met all the criteria of the protocol and provided consent and data for full semen analysis. Four men withdrew from the study, leaving 92 infertile male partners who completed 3 months of antioxidant supplement (Profertil®) treatment and 103 sub-fertile men who did not take any supplement. The latter served as the control group. The mean (SD) age of infertile male partners taking the antioxidant supplement (Profertil®) was 37.04 (6.83), whereas in the control group it was 35.30 (5.40). Table 1 summarizes the demographic characteristics of the population by both groups.

Table 1 shows the baseline demographic characteristics between the control and treatment groups (i.e., male partners receiving Profertil® treatment). Our data demonstrated that the mean age of the husband and etiology of infertility were significantly different ($p < 0.05$) between treatment and control groups. However, a more recent study found that the decline in sperm parameters did not begin until after 50 years of age (Pino et al., 2020). We observed that younger infertile men in the treatment group (mean age = 35.30 years old \pm 5.40) as compared to the control group (mean age = 37.04 \pm 6.83) ($p = 0.02$), indicating the possibility of younger infertile men were more open to seeking for fertility treatment. When comparing the etiology of infertility, the proportion of male partners classified in the male factor category and treated with Profertil® was higher (70%), as compared to the male partner categorized in the combined factor category

(30%) ($p = 0.02$) (data not shown). Our data suggested that male partners classified in the male factor category were more likely to receive Profertil® treatment to increase sperm quality as compared to those male partners that have female partners with infertility conditions.

Figure 1 shows the results of the improvement of sperm parameters after three months of antioxidant (Profertil®) supplement intake. When compared to their baseline sperm quality, men receiving Profertil® had highly significant increases in median sperm concentration concentration ($p < 0.0001$), motility ($p < 0.0001$), progressive motility ($p < 0.0001$), total sperm count ($p < 0.0001$), total sperm motility ($p = 0.01$) and total progressive motility ($p < 0.0001$). Moreover, sperm abnormality of a total of 24 (26.1%) men improved after receiving Profertil® ($p = 0.013$).

Discussion

The respondents in Profertil® group showed a positive effect and statistically significant difference in sperm parameters after three months of treatment except for semen volume where we did not observe any changes between the control and treatment groups ($p = 0.943$). The improvement was seen in Profertil® group resulting by taken 2 daily capsules of Profertil® in 3 months. This is because each capsule of the antioxidant supplement intake (Profertil®) contained: L-carnitine (440 mg), L-arginine (250 mg), zinc (40 mg), vitamin E (120 mg), glutathione (80 mg), selenium (60 mg), coenzyme Q10 (15 mg), and folic acid (800 mg) that be used as an antioxidant supplement among OATs infertile male. Each ingredient has a beneficial effect on fertility, in particular on sperm quality, and is therefore recommended as a potentially effective therapy for the treatment of male infertility (Lipovac et al., 2016; Lipovac et al., 2014; Imhof et al., 2012).

Many previous studies also have shown the positive effect of mono-micronutrients which act independently. Carnitines (L-carnitine (LC) and L-acetyl carnitine (LAC) provide energy substra-

Table 1: Demographic and baseline characteristics of the study population (n= 95), stratified

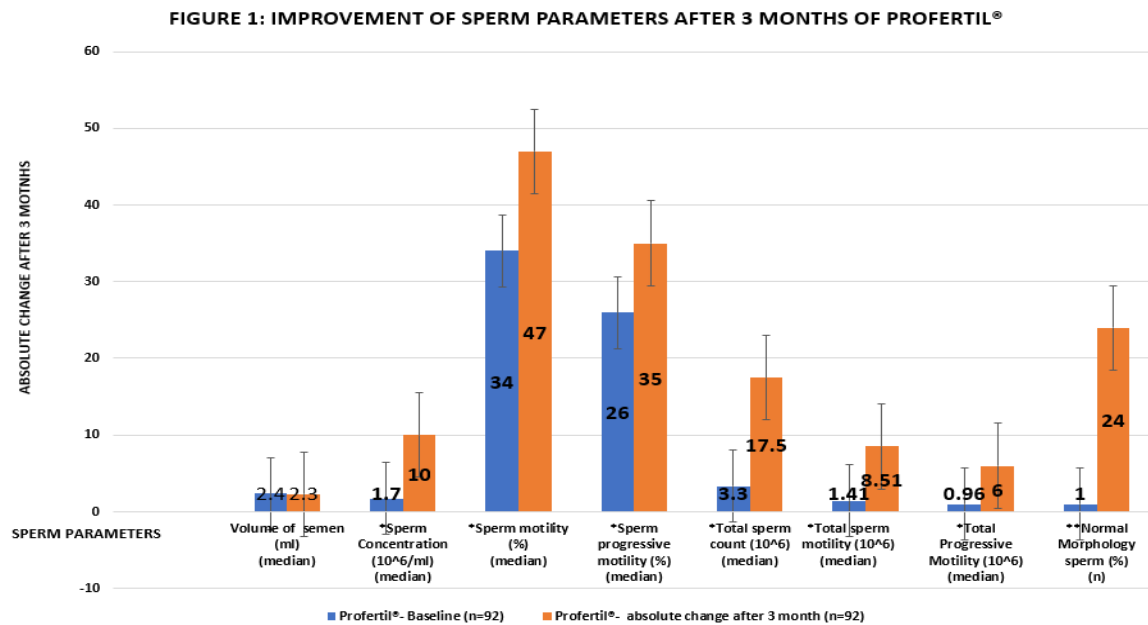
by antioxidants supplement intake group (Profertil®).

CHARACTERISTICS	CONTROL ^a (N=103)	TREATMENT ^D (N=92)	P-VALUE
Age of wife (mean, SD)	34.07 (4.45)	33.05 (3.67)	0.08
Age of husband (mean, SD)	37.04 (6.83)	35.30 (5.40)	0.02*
Ethnicity			
Malay (n,%)	64 (62)	59 (64)	0.96
Chinese (n,%)	15 (14.6)	13 (14)	
Indian (n,%)	24 (23.3)	20 (22)	
Etiology of infertility			
Male (n,%)	56(54)	64(70)	0.02*
Combined (n,%)	47(46)	28 (30)	
Husband BMI			
Underweight (n,%)	1(1)	1 (1)	0.11
Normal (n,%)	34 (33)	19 (21)	
Overweight (n,%)	49 (48)	43 (47)	
Obese (n,%)	19 (18)	29 (32)	
Wife BMI			
Underweight (n,%)	2(2)	4(4)	0.65
Normal (n,%)	48 (47)	47(51)	
Overweight (n,%)	45(44)	34(37)	
Obese (n,%)	8(8)	7(8)	
Smoking			
Yes (n, %)	12(12)	16(17)	0.25
No (n, %)	91 (88)	76(83)	
Primary/Secondary fertility			
1° fertility (n, %)	95(92)	92(100)	NA
2° fertility (n, %)	9(8)	0	

*Significant differences as compared between different groups at p<0.05

*Independent variables were presented as demographic and baseline characteristics (i.e age of wife/husband, ethnicity, etiology of infertility, husband/wife BMI and primary/secondary fertility).

*Dependent variables were presented as the control or treatment (i.e outcomes).



Data are expressed as median (IQR) and analysed using Kruskal-Wallis test.
Data are expressed as frequencies (%) and analysed using Chi-square test.
*Significant difference between the baseline and after treatment group $p_{\text{adjusted}} < 0.05$.
**Significant difference between the baseline and after treatment group $p < 0.05$

ates of spermatozoa and thus, are important in sperm metabolism fuelling important activities like sperm motility. Banihani et al. (2014) reported in vitro studies of sperm cultured in media containing carnitines had higher motility and viability in comparison with controls. Abd-Elrazek and Ahmed-Farid (2018) showed that the administration of L-carnitine to adult oligospermic rats attenuated the cytotoxic effect of busulfan by improving sperm morphology, motility, velocity, and count. Moreover, Nazari et al. (2021) proved that antioxidant supplementation containing 1500 mg of L-carnitine can improve sperm quality in infertile men by improving cell concentration and overall motility.

A study by Wang et al. (2014) indicated that nitric oxide (NO) had statistically significant positive impacts on sperm viability and motility. Arginine acts as an immediate precursor of NO. Jihad Manssor et al. (2019) reported that L-arginine showed a highly significant increase in total sperm count, motility and ejaculate volume after treatment with L- Arginine. In coenzyme Q10 (CoQ10), some previous studies reported

that mean sperm concentration (Alah, 2019, Lafuente et al., 2013; Safarinejad et al., 2012), motility percentage (Alah, 2019, Cakiroglu et al., 2014, Lafuente et al., 2013, Safarinejad et al., 2012), total sperm count (Safarinejad et al., 2012) and percentage of sperms with normal forms (Cakiroglu et al., 2014; Nadjarzadeh et al., 2014; Safarinejad et al., 2012) improved significantly after CoQ10 administration. The latest study revealed oral CoQ10 as an effective treatment for improving the sperm parameters (motility, morphology, sperm concentration) which were statistically significant except for semen volume where p value was > 0.05 (Saeed Alkumait et al., 2020).

Matorras et al. (2020) reported a statistically significant increase in progressive motility in the vitamin E group compared with before-treatment values. Another study showed that vitamin E combined with clomiphene citrate, as an antioxidant and anti-estrogen therapy, was more efficient in improving sperm concentration in idiopathic OAT compared with each one individually (EISheikh et al., 2015).

Zinc also plays an important role in testicular development and sperm formation (Fallah et al., 2018; Chemek et al., 2016). Some previous studies revealed that zinc supplementation could significantly increase sperm volume (Zhao et al., 2016), sperm motility (Alsalman et al., 2018, Zhao et al., 2016), and percentage of normal sperm morphology (Zhao et al., 2016) of infertile men. In an animal study, obese rats treated with zinc showed an increase in sperm count, sperm motility, and testosterone levels and thus improved testicular structure and spermatogenesis abnormalities (Ma et al., 2020). Folic acid is also known as vitamin B9 and play role in the nucleic acid synthesis and amino acid metabolism. Folic acid is required for DNA synthesis (Balashova et al., 2018) and is thus important for spermatogenesis (Ly et al., 2017; Yuan et al., 2017). Azizollahi et al. (2013) claimed that folic acid administration could increase sperm number. Hence, zinc sulfate was better than folic acid when the change in morphology was reported.

Glutathione is a tripeptide composed of glutamate, cysteine, and glycine and is the most abundant non-thiol protein found in mammalian cells. It acts as an intracellular defense against ROS in mammalian cells by forming a protective layer on the spermatozoa plasma membrane (Sinha and Gupta, 2018; Kolesnikova et al., 2017) and is required for DNA synthesis (Saeed Alkumait et al., 2019). Opuwari and Henkel (2016) claimed the low level of glutathione ultimately unstable the middle portion of the sperm which affects the motility rate of sperm. Some previous studies revealed the positive effects of glutathione supplementation on sperm motility and morphology (Saeed Alkumait et al., 2020, Saeed Alkumait, 2019; Majzoub and Agarwal, 2018) as well as sperm concentration (Saeed Alkumait et al., 2020, Saeed Alkumait, 2019).

Mossa et al. (2018) reported a significantly increase in sperm count, motility, viability, normal sperm morphology, and ejaculate volume in infertile men compared to after treatment with selenium capsule (50 microgram). In a combination antioxidant consisting of Folic acid (5 mg), Selenium (200 ug) and Vitamin E (400 iu), Zadeh et al. (2019) studied showed statistically significant differences in sperm count ($p = 0.031$) and

motility ($p = 0.01$) after six months treatment compared with the control group. In an animal study, Stefanov et al. (2018) reported selenium supplementation in the main diet can be used for improving the sperm parameters in rams which showed a positive influence on the ejaculate volume, sperm motility, and sperm survival rate after incubation at 39°C for 360 minutes, without negative effect on pH of the ejaculates.

Our findings supported by previous studies indicated that the use of an antioxidant supplement (Profertil®) showed a significant improvement in semen analysis of sub-fertile men after treatment (Lipovac et al., 2016 and Lipovac et al., 2014) and led to a 25.8% pregnancy rate (Imhof et al., 2012). Lipovac et al. (2016) reported that volume, density, motility, overall progressive motility, and sperm morphology significantly improved after 3 months of treatment in Profertil® and L- carnitine groups as compared to baseline ($p < 0.001$). This study also claimed that relative change for sperm density and overall progressive motility was found to be higher for the combined micronutrient treatment groups (Profertil®) as compared to the mono treatment group using L- carnitine alone ($p < 0.05$). Lipovac et al. (2014) also reported that 67 subfertile males after being treated with Profertil® demonstrated a significantly higher increase of sperm hyaluronan acid-binding (74.6% vs 30%, $p = 0.001$). This sperm hyaluronan acid-binding is used to indicate sperm maturation which is of importance for enabling fertilization, increases the probability of natural conception or ART pregnancy, and thus helps to reduce miscarriages and birth defects rate. Imhof et al. (2012) also proved that 132 of 205 subfertile men treated with Profertil® showed improvement in all sperm parameters such as volume of semen, sperm concentration, motility, and morphology as well as the pregnancy when compared to the control group. Schauer (2011) demonstrated that post-3 months of Profertil® treatment evaluation resulted in improvement of all sperm parameters in men with a 2-year history of unexplained subfertility and subclinical varicocele and the occurrence of pregnancy in 41.18% after 6 months follow-up. Profertil® improved all sperm fertility-relevant parameters and interestingly, a total of 30 of 120 subjects achieved a normal sperm count without any abnormalities and 21 pregnancies have been

reported by Imhof et al (2010). However, other studies did not report this outcome (Steiner et al., 2020, Joseph et al., 2020) even though the dosage of micronutrient ingredients, number of mono-micronutrients, and brands are differently used as antioxidant micronutrient supplements in their study, its acts in combination micronutrients.

Another previous study on combination micronutrients called FH PRO for Men (Fairhaven Health LLC, Bellingham, WA, USA) consists of vitamin A (as beta-carotene): 5000 IU, vitamin C: 120 mg, vitamin D3: 1200 IU, vitamin E (as mixed tocopherols): 200 IU, vitamin K: 80 µg, thiamin: 3 mg, riboflavin: 3.4 mg, niacin: 20 mg, vitamin B6: 25 mg, folate: 800 µg, vitamin B12: 1000 µg, biotin: 600 µg, pantothenic acid: 20 mg, iodine: 150 µg, zinc: 30 mg, selenium: 140 µg, copper: 1 mg, manganese: 2 mg, chromium: 120 µg, molybdenum: 75 µg, L-carnitine tartrate: 2000 mg, L-arginine: 350 mg, CoQ10: 200 mg, N-acetyl L-cysteine: 200 mg, grapeseed extract: 20 mg, lycopene: 10 mg and benfotiamine: 1 mg. This study showed a statistical improvement in sperm concentration, total motility, progressive motility, and normal morphology which supported our current finding (Arafa et al., 2020). Most of the above-published studies prove the treatment of male infertility with antioxidant supplementation can neutralize ROS and thus, improve sperm quality and lead to pregnancy (Son and Gadallah, 2018; Majzoub and Argawal, 2018; Lipovac et al., 2016; Lipovac et al., 2016; Showell et al., 2015; Lipovac et al., 2014; Imhof et al., 2012). The Cochrane meta-analysis 'Antioxidants for male subfertility' was recently updated and includes 61 randomized controlled trials and 6264 infertile men and concluded that antioxidant therapy was associated with sperm parameters as well as an increase in clinical pregnancy rate (CPR) and live birth rate (LBR) (Smits et al., 2019).

Furthermore, all improvements in the treatment group (Profertil®) could also result in a change in lifestyle habits such as a reduction in nicotine consumption (smoking), nutrition, and physical activity. The male partners in this study practice a healthy lifestyle as suggested by our clinicians and embryologists. In conclusion, our data showed Profertil® has a positive effect on semen parameters in men with OAT

Conclusion

Our data showed Profertil® has a positive effect on semen parameters in men with OAT.

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