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COMBINATION OF MACA, KOREAN GINSENG EXTRACT AND ANTIOXIDANT THERAPY FOR MALE WITH OLIGOASTHENOZOOSPERMIA: CASE STUDY

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Abstract

More than 70 million couples suffer from infertility worldwide. Infertility is defined as not being able to get pregnant despite having frequent, unprotected sex for at least a year. Extraordinary advances have been achieved in the field of male infertility in the last decades. In recent years, the use of antioxidants in treatment of infertile men has been suggested, although there is limited evidence about the influence of nutrition on quality of semen. In this cohort study we aimed to evaluate the effect of Maca, Korean ginseng extract and antioxidants (vitamin C, natural vitamin E, zinc, selenium, L-Arginine, L-Carnitine, L-Methionine and L-Phenylalanine) on a male previously diagnosed with oligoasthenozoospermia, incapable to achieve fertilization of the healthy spouse.

The man at age of 30, from Skopje, was supplemented daily with Maca tablets 500 mg three times a day, and once a day with a tablet under a brand name available in Macedonia, consisted of Korean ginseng extract and antioxidants mention above, in a period of four months, from March to July 2017. Semen analysis with microscope phase-contrast was done in andrology laboratory in the policlinic "Bukurest", with 3 days of abstinence was done before starting with the therapy and after.

First analyze showed 20% of motile spermatozoa. Second analyze was done 41 days later and showed improvement of motile spermatozoa to 35%, while the last semen analysis was done 83 days after the second sample, which showed significant improvement of motility (50% which meets the criteria of the WHO), morphology, concentration and total number of spermatozoids that classified the man as normozoospermic.

We assume that, Maca, Korean ginseng extract and antioxidants intake could improve the quality of semen parameters in men with oligoasthenzoospermia and increase fertility rate.

***Key words**: Male infertility, Antioxidants, Semen quality, Oligoasthenozoospermia.*

1. Introduction

Male infertility is a worldwide healthcare issue, which has driven developed countries to take the necessary steps in detecting and treating the problem. While transition countries such as Macedonia and most of the surrounding states belonging to the Western Balkans, where cultural differences and patriarchal societies, usually blame the female partner for infertility. Thus, male factor infertility is underestimated. Because of this, accurate statistics may be prevented from being collected and compiled and that's why in Macedonia we still don't have an accurate cipher of couples that experience infertility in their reproductive ages, neither a cipher of infertile males. Male infertility contributes to approximately half of all cases of infertility and affects around one in 20 men in the reproductive age group (defined here as between puberty and

40 years of age) [1]. The global incidence of infertility is about 15% [2], and is defined as incapability of a man to make a fertile woman pregnant [3] after twelve months of frequent, unprotected sex [4].

Normal reproductive function is vital for producing offspring and sexual satisfaction in man. Diagnosing male infertility problems usually involves: physical examination, semen analysis, hormone tests, testicular biopsy, urine test, etc. There are different pharmacological, non-pharmacological, combination and ethno-pharmacological treatment options for male infertility [5]. Semen analysis may identify and characterize the following impairments in male: oligozoospermia (low concentration of sperm), asthenospermia (reduced sperm motility), teratozoospermia (sperms with abnormal morphology), and the combination of all of them (oligoasthenoteratozoospermia) [6].

The last 25 years has seen a phenomenal advance in the field of knowledge about male infertility mainly due to the increased understanding of reactive oxygen species (ROS), oxidative stress (OS) and technological innovations. Low levels of ROS are required for several redox-sensitive physiological processes, such as sperm capacitation and hyperactivation, although supraphysiological ROS levels impede sperm membrane fluidity and permeability [7]. Normally equilibrium exists between ROS production and antioxidant scavenging activities in the male reproductive tract [8]. Seminal fluid is rich in antioxidants that nourish and protect the sperm. They exist in two forms; an enzymatic and a non-enzymatic antioxidant system [9]. Evidence show lower antioxidant capacity of semen in infertile men that explain the reduced semen antioxidants and high levels of ROS compared to fertile men [10, 11]. Thus, proper understanding of the pathophysiology is very important for effective treatment algorithm.

Given the difficulty of reaching an accurate diagnosis, many antioxidant therapies have been used in the hope of improving sperm quality [12]. This has led to the development of various antioxidants. Antioxidants are compounds that are acquired through ingesting a balanced diet or from oral supplements [13]. While antioxidant supplementation has been proposed as an approach to increase the scavenging capacity of seminal plasma [14], controversy still surrounds.

Multiple randomized trials have investigated antioxidant supplementation for treatment of male factor infertility [15 - 17], with several demonstrating a positive effect on semen quality, especially sperm motility [18], and improve the rates of fertilization and pregnancy outcomes [19]. Among all available antioxidants, the most frequently prescribed compounds include vitamins E and C, carnitine, N-acetyl cysteine (NAC), selenium (Se), and zinc (Zn) [14].

The empiric use of antioxidants for patients with oligoasthenozoospermia (OA) is aimed at improving semen parameters like concentration and total motility and thereby increasing the probability for conception. Currently, multi-antioxidant supplementations are considered as an effective therapy for male infertility. The synergetic effect of multi antioxidants combinations made them attractive for most of the researchers of male infertility scope.

In this cohort case study, we aim to investigate the rationale behind using maca (*Lepidium meyenii*), Korean ginseng extract and antioxidants (vitamin C, natural vitamin E, zinc, selenium, L-Arginine, L-Carnitine, L-Methionine and L-Phenylalanine) on a male previously diagnosed with OA, and the evidence surrounding their clinical utility. Clinical pregnancy outcome rate was not considered to be the main outcome and was excluded from the analysis.

2. Materials and Methods

2.1 Study protocol

The investigation was cohort prospective study carried out from March to July 2017, 125 days exactly. Before commencing the study, patient obtained written informed consent and verbal explanation of the nature of the study. One evaluation of spermiogram was performed before antioxidant intake, one in April and the last consecutive semen sample was collected in July (semen parameters are presented in Tables 3 and 4). The liquefied semen sample was assessed for the following parameters: Semen volume (mL), pH, agglutination, viscosity, sperm concentration (million/mL), sperm progressive motility (%), total sperm motility (%), and normal sperm morphology (%). All samples were evaluated with microscope phase-contrast at andrology laboratory in the policlinic "Bukutest" Skopje, with 3 days of abstinence.

The study was conducted in line with European Urology and Good Clinical Practice guidelines, with ethical principles laid down in the latest version of the Declaration of Helsinki.

2.2 Composition of antioxidant formulation tablet

Supplementation of the patient was done with to tablets: one tablet consisted of the active maca substance taken 3 times daily, and the other tablet was a combination of Korean ginseng extract, vitamin C, vitamin E, zinc, selenium, L-Arginine, L-Carnitine, L-Methionine and L-Phenylalanine, available in Macedonia under a brand name, manufactured in United Kingdom, taken 2 times daily (see Table 1 for dosage of each

component). The subject received this two tablet combination in a period of 4 months.

The following antioxidants were selected based on the available evidence for their use, particularly in infertile men with OA. Why we were determined for this combination of antioxidants, it is because of their scientifically acceptable evidence demonstrating benefit in improving semen parameters. In Table 2 is shown their daily dosage, protective mechanism, effect, reference and level of evidence based.

Table 1. Content and dosage of maca, Korean ginseng extract and antioxidants

Tablet content (average)	Amount	% Nutrient Reference Value (NRV)
Maca (*Lepidium meyenii*) powder (5 : 1) (≡ to 500mg maca powder)*	500mg	***
Korean ginseng extract (15 : 1) (≡ to 900 mg Korean ginseng powder)**	60mg	***
Vitamin C**	100mg	125
Natural vitamin E (100iu)**	67mg	558
Zinc**	15mg	150
Selenium**	200ug	364
L-Arginine**	250mg	***
L-Carnitine**	50mg	***
L-Methionine**	50mg	***
L-Phenylalanine**	50mg	***

Legend: *: Maca substance used as single tablet; **: Combination of antioxidants composing the second tablet; ***: EC Nutrient reference value not yet established.

Table 2. Mechanism and effect of antioxidants in prescribed in daily dose. Level of evidence surrounding their effect

Compound	Daily dose	Protective mechanism	Reported effect	Ref.	LE
Maca (*Lepidium meyenii*)	1,500 mg	↑ number of sperm released during spermiation. Neurobiological activity of antioxidant protection	↑ LH, sperm concentration, total sperm count, and count of motile sperm	[20 - 26]	B
Korean ginseng extract	120 mg	Induce spermatogenesis via CREM activation	↑ total and free testosterone, DHT, FSH and LH levels, motility and total number of sperm. ↓ in mean PRL	[27 - 31]	B
Vitamin C	200 mg	Neutralising free radicals	↓ percentage of sperm DNA fragmentation. ↑ sperm motility.	[32 - 34]	B
Vitamin E	134 mg	Neutralizes free radicals and protects cellular membrane against O_2 free radicals	↑ in sperm concentration and motility ↓ of oxidative stress measures and SDF	[35 - 38]	A
Zinc	30 mg	Anti-apoptotic, anti-bacterial and antioxidant properties, prevents damage to chromosomes	↑ all semen parameters and spontaneous pregnancy rates	[39 - 41]	B
Selenium	400 ug	Help maintain normal sperm structure integrity	↑ all semen parameters and ↓ of OS measures	[4, 18, 42, 43]	B
L-Arginine	500 mg	↓ lipid peroxidation of spermatozoa	↑ sperm count, motility ↓ erectile dysfunction	[48 - 50]	C
L-Carnitine	100 mg	Considered as fuel source	↑ motility and viability	[14, 44 - 47]	A
L-Methionine	100 mg	Antioxidant defense mechanism by reacting readily with oxidants to form methionine sulfoxide	↑ motility and morphology ↓ reduction of SDF	[51 - 52]	C
L-Phenylalanine	100 mg	Contributing to the stability of DNA methylation	retains sperm integrity and vitality	[53]	NE

Legend: LE is level of evidence is modified from the Oxford Centre for Evidence-based Medicine [58]. Grade A is based on systemic reviews of randomized controlled trials (RCT's) or individual RCT of good quality; Grade B is based on well-designed studies (prospective, cohort) and lower quality RCT; and Grade C is based on poorer quality studies (retrospective, case series, and expert opinion). NE= No Evidence; SDF - Sperm DNA fragmentation; OS - Oxidative stress; RCT - Randomized controlled trial; CREM - cAMP-responsive element modulator; DNA - Deoxyribonucleic acid; DHT - Dihydrotestosterone; LH - Luteinizing hormone; FSH - Follicle-stimulating hormone; PRL - Prolactin.

2.3 Literature research strategy

The search strategy was modified to comply with the requirements of each database consulted. We performed an exhaustive meticulous electronic research in the following databases (from January 1990 until March 2018): Medline database using PubMed, Cochrane library, EBSCOhost, Researchgate, and Medscape. The search combined terms and descriptors related to male infertility, antioxidants, semen parameters and oligoasthenoteratozoospermia (OAT), and justify the use of antioxidant in improving semen parameters. Animal and laboratory studies were excluded from research strategy.

2.4 Statistical analysis

Computations were performed using the statistical software package IBM®SPSS® Statistics, for Windows, Version 24. Paired Student's t-test was used to compare seminal fluid parameters before and after supplement; $p < 0.05$ was considered statistically significant. Microsoft Excel 2010® was used for designing charts.

3. Results and Discussion

3.1 Case Presentation and laboratory outcomes

Patient 30 years old from Skopje (profile showed in Table 3), reported in a primary health care organization his inability of having offspring. Although they tried to conceive, they could not succeed after two years of regular, unprotected sexual intercourse. His wife underwent before, all the required investigations and the results showed that she was able to conceive pregnancy.

After evaluation of the first spermiogram (semen parameters reported in table 3), patient was diagnosed with OA.

Differences between semen parameter at the beginning, after 41 days of treatment and at the end of receiving antioxidant supplements are shown in Table 4.

According to the criteria included in the 5th edition of the World Health Organisation (WHO) 2010, manual, a man has OA when less than 15 x 10^6 spermatozoa per millilitre (mL) and less than 32% of sperm in

Table 3. Profile of the patient and semen parameters before receiving antioxidant therapy

Patient Profile		Semen parameters		
Age	30	**Analyse**	**Result**	**Ref. values**
Address	Skopje	volume (mL)	**3**	> 2 mL
Profession	Freightage	pH	**8**	≥ 7.2
BMI	28.41	Agglutination	**No**	No
Smoking	Yes	Viscosity	**Normal**	Normal
Alcohol	Weekends	Concentration (x 10^6 mL)	**10 (x 10^6 mL)**	>20 (x10^6 mL)
Narcotics	No	Progressive motility (%)	**10%**	>25 %
Coffee	Excess	Non-motile (%)	**60%**	No lower limit
Fast food	Very often	Total motility (%)	**20%**	> 50%
Family history	No	Morphology (%)	**44%**	> 30%
	Diagnose	**Oligoasthenozoospermia**		

Table 4. Ejaculate parameters before in the middle of treatment and after treatment with Maca, Korean Ginseng extract and antioxidants

Parameters	Before	Middle	After	Significance (p-Value)
Volume (mL)	3.0	3.0	2.5	**ns ***
pH	8.0	8.0	8.0	**ns***
Agglutination	No	No	No	**ns***
Viscosity	Normal	Normal	Normal	**ns***
Concentration (x 10^6 mL)	10 (x 10^6 mL)	25 (x 10^6 mL)	25 (x 10^6 mL)	**ρ < 0.05****
Progressive motility (%)	10%	20%	25%	**ρ < 0.05****
Non-motile (%)	60%	40%	25%	**ρ < 0.05****
Total motility (%)	20%	35%	50%	**ρ < 0.05****
Morphology (%)	44%	48%	50%	**ρ > 0.05*****

Legend: *There was no significance before and after treatment with the antioxidant formula; **There is a significant effect of antioxidant formula before and after treatment; ***Low impact on improving sperm morphology but not statistically significant.

the ejaculate have progressive motility (sperm which move around) or when there it less than 40% total motile sperm (motile sperm which do and do not move around).

The andrology laboratory in policlinic "Bukutest" in Skopje still uses the WHO manual criteria published in 1999 which says that a man has OA when less than 20 x 10^6 spermatozoa per mL and less than 25% spermatozoa in the ejaculate have progressive motility, or less than 50% of total motile sperm.

In Table 4 are shown changes in sperm concentration, total sperm motility, progressive motility and non-motile sperm. We mention only this parameters because there is a significant change ($\rho < 0.05$) only in this parameters before and after finishing with the antioxidant therapy.

These improvements and their significance before, in the middle and after receiving the antioxidant are shown in Figures 1, 2 and 3.

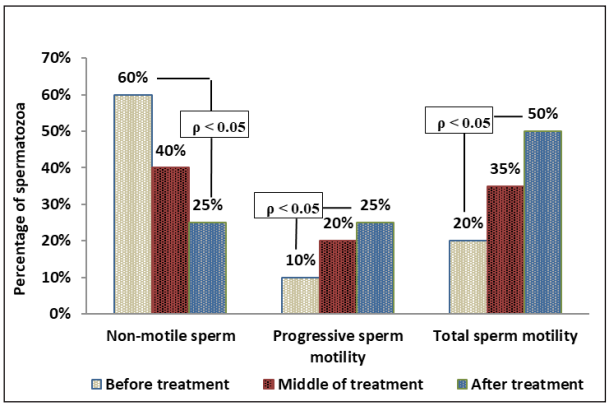

Figure 1. Changes in sperm motility before, in the middle and after antioxidant therapy

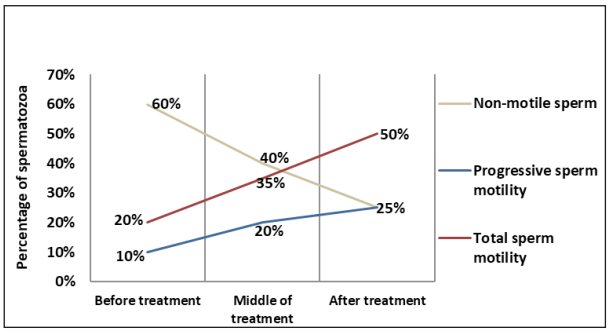

Figure 2. Differences in sperm motility before, in the middle and after antioxidant therapy

The current study describes the possible positive effect between intake of: maca, Korean ginseng extract, vitamin C, vitamin E, zinc, selenium, L-Arginine, L-Carnitine, L-Methionine, L-Phenylalanine and oligoasthenozoospermia using a case study.

In the studied patient, after 125 days of receiving this combination of therapy, we report improvements in: sperm concentration, total sperm motility, progressive motility, and it is obvious the decrease in percentage of non-motile sperm. Concentration of sperm before treatment was 10 x 10^6 per mL.

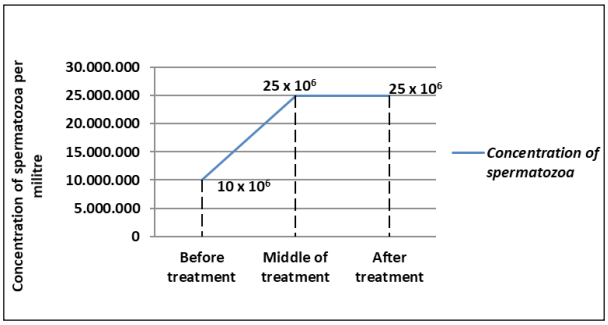

Figure 3. Concentration of sperm before in the middle and after antioxidant therapy

After 41 days of receiving 1500 mg dose of maca and the tablet consisted of antioxidant mentioned in Table 1 (2 times daily), sperm concentration improves to 25 x 10^6 per mL and remains the same until the end of treatment, 84 days exactly. Significant improvements can be seen in progressive and total sperm motility. Progressive motility increases from 10% before treatment, to 20% after 41 days and became 25% by the end of the treatment. Furthermore, total sperm motility improves from 20% before treatment, to 35% in the middle of treatment and becoming 50% by the end of the therapy, which qualifies this man as normozoospermic. It is interesting to mention the decrease percentage of non-motile spermatozoa from 60% before treatment, to 40% in the middle, and 25% after 125 days of double combination therapy.

Oxidative stress is not the cause of male infertility in all patients. The rationale for antioxidant therapy in infertile patients should be based on raised OS status [54]. According to Ko E.Y *et al.,* there are no existing standards as to the optimal oral antioxidant supplement regimen, the dosing of specific agents, or the duration for administering supplements [55]. However, given the rather innocuous side effects at or below the recommended daily allowances, oral antioxidant supplementation may be a reasonable treatment regimen before proceeding with more expensive treatments such as in vitro fertilization or Intracytoplasmic sperm injection [56].

Although, we didn't have in the combination Coenzyme Q10 (CoQ10), N-Acetyl cysteine (NAC), folic acid and lycopene, which are verified in a number of studies that have beneficial effects on semen parameters, the combination of antioxidants used for this case replenish the necessary requirements for improving sperm

concentration and sperm motility. Anyway, further randomized controlled trial on larger sample size for standardization of doses and duration of supplementation are needed. The patient selection is one of the important aspects to be considered.

Unfortunately, many drugs are currently used without any rationale: such therapies are often any improvements that do occur in semen parameters may be due to other unrelated reasons. Furthermore, the available forms of treatment have mostly produced only marginally satisfactory responses, even in the best of proper trials [57].

4. Conclusions

- Considering cost-efficiency of antioxidant administration to infertile men, the potential advantages that such treatment offers cannot be ignored.

- This case study is an encouragement for further research involving a larger sample size, which can help in determining the appropriate antioxidant compounds as well as certain dose of antioxidants..

- Moreover the future studies should concern the pregnancy rate as a primary outcome.

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