



Double-Blind, Randomized, Placebo-Controlled Clinical Trial Evaluating the Effect of Antioxidants on DNA Fragmentation Index in Men with Idiopathic Oligoasthenoteratozoospermia

Dr. Ameet Patki ^{*1}, Mr. Rohit Shelatkar ², Dr. Monica Singh ³, Dr. Sweta Agarwal ⁴, Dr. Venugopal M. ⁵,
Dr. Shashikant Umbardand ⁶, Dr. Apoorva Reddy ⁷, Dr. Priya Kannan ⁸, Dr. Srilatha Gorthi ⁹,
Dr. Gautam Khastgir ¹⁰, Dr Anita Kulshreshtha ¹¹, Dr. Gayatri Ganu ¹²

¹Medical Director, Fertility Associates, 4th Floor Gupte House, Near Khar Police Station, 81, S V Road, Khar West, Mumbai, Maharashtra, India.

²Senior Vice President, Director, Vitabiotics Ltd, 1 Apsley Way, London, NW2 7HF, UK.

³Consultant, Bhopal Test-Tube Baby Centre, E-1/13A, Arera Colony, Bhopal, India.

⁴Director, Southern Gem Hospital, 5-9-30/1/7, Gowri Parmeshwari Mansion, Palace Colony, Basheerbagh, Hyderabad, India.

⁵Executive Director, ARMC IVF Fertility Centre, Ayyanthole, Thrissur, India.

⁶Director and Consultant, Mangalya Nursing Home & Atharva Fertility Center, 738, 739 and 740, South Kasba, Near Kali Masjid Solapur North, Solapur, Maharashtra, India.

⁷Consultant, Phoenix Speciality Clinic, 1st Floor, no 72 TSN Arcade, 28th MR, 9th Block Jayanagar, Bangalore, India.

⁸Director & Embryologist, Garbba Rakshambigai Fertility Centre, 4, 6th Cross Street, United India, Kodambakkam, Chennai, Tamil Nadu, India.

⁹Consultant, Revive Clinic, Concrete Cornet, Huda Trade Centre, Serilingampally, Hyderabad, Telangana, India.

¹⁰Medical Director, Bengal Infertility & Reproductive Therapy Hospital (BIRTH), 36B, Elgin Road, Kolkata, India.

¹¹Consultant, Yashlok Hospital, 43A/31A Hashimpur Road, PrayagRaj, India.

¹²Director, Mprex Healthcare Pvt. Ltd. Office Number 501, 514 Crossroads, Bhumkar Square, Wakad, Pune, India.

*Corresponding author: Dr. Ameet Patki; ameetpatki@fertilityassociates.in

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Abstract

Introduction: Oxidative stress, sperm apoptosis, and DNA fragmentation are significant factors in male infertility. Sperm DNA damage is associated with reduced fertility and increased frequency of spontaneous abortions and affects embryo quality. Spermatogenesis is an energy-intensive process, it requires a highly balanced supply of minerals, antioxidants, and nutrients. Hence, food supplementation is thought to be a potential therapeutic option that may improve seminal fluid conditions, provide energy to male germ cells, and protect them from oxidative stress.

Methodology: A randomized placebo-controlled clinical trial was conducted with the Oligocare Forte Plus tablet in treating oligospermia in subfertile males. Reduction in DNA fragmentation index % (DFI) was assessed at baseline and day 90 along with the pregnancy incidences amongst the couple enrolled for study. **Results:** Subjects with initially elevated DFI demonstrated a significant decrease post intervention. Among those with DFI >20%, Oligocare Forte Plus group witnessed an 18.63% reduction versus 12.63% in the placebo group. For DFI >40%, the decrease was 44.84% with Oligocare Forte Plus compared to 26.87% with placebo. The Oligocare Forte Plus group exhibited more incidence of pregnancy. Notably, there were no instances of premature subject discontinuation. Throughout the study, no adverse events or abnormalities were reported, indicating the safety and favorable tolerance of the Oligocare Forte Plus tablet. **Conclusion:** The current study serves as a confirmatory examination of the efficacy of Oligocare Forte Plus for the treatment of Oligoasthenoteratozoospermia, aiming to establish its viability as a therapeutic option before considering Intrauterine Insemination, Assisted Reproductive Technologies, or In Vitro fertilization procedures.

Keywords: DNA damage, DFI, Oligospermia, nutritional supplement, Oligocare

Introduction

Around 17.5% of the adult population - roughly 1 in 6 worldwide - experience infertility, showing the urgent need to increase access to affordable, high-quality fertility care for those in need. In 2019, the global prevalence of male infertility was estimated to be 56,530.4 thousand (95% UI: 31,861.5-90,211.7), reflecting a substantial 76.9% increase since 1990 [1].

Surgical methods (e.g., varicocelectomy), hormonal and pharmaceutical therapies (e.g., clomiphene citrate or hCG), intracytoplasmic sperm injection (ICSI), assisted reproductive technologies (ART), Intrauterine Insemination (IUI), In Vitro fertilization (IVF), and ICSI are all viable therapeutic options for male infertility [2]. Each of the mentioned therapeutic methods comes with specific challenges, such as limitations in efficacy, increased treatment expenses, uncertainty about clinical effectiveness, and the possibility of adverse consequences.

Approximately 15% to 40% of men are infertile despite having normal sperm parameters, normal medical history, and normal physical examination; overall, this condition is currently defined as unexplained male infertility (UMI) [3].

Spermatogenesis demands an exceptionally well-balanced provision of nutrients, minerals, and antioxidants due to its high energy demand. Therefore, it is hypothesized that food supplementation might serve as a viable therapeutic approach to enhance the condition of seminal fluid, sustain male germ cells with energy, and shield against oxidative stress [4].

Prostate apoptosis, Deoxyribonucleic acid (DNA) fragmentation, and oxidative stress all play substantial roles in the

pathophysiology of male infertility. Damage to sperm DNA is linked to decreased fertility, an increased incidence of spontaneous miscarriages, and a decline in the quality of the resulting embryo [5]. Furthermore, the DNA fragmentation index (DFI) is well recognized as a possible indicator of fertility, and its identification has been documented several times as a predictive factor for male fertility in a variety of assisted reproductive technologies (ART) [6].

Numerous international studies have confirmed that nutrition does have a role in male factor infertility. Additionally, some dietary deficits are strongly associated with male infertility. Supplementing diet is a risk-free method for enhancing semen quality indicators and minimizing DNA damage to sperm [7].

Oligocare Forte Plus is a nutraceutical comprising a balanced antioxidant blend. We propose to validate the effectiveness of this intervention on DFI in subjects with idiopathic Oligoasthenoteratozoospermia

Materials and methods

Study design

We conducted a prospective randomized controlled trial involving patients with oligospermia recruited from the outpatient department of respective study sites. The study was conducted at ten different sites across India. The study was approved by the Royal Pune Independent Ethics Committee, Pune, Maharashtra, and was registered with the Clinical Trial Registry of India (CTRI/2020/12/029590). The consolidated standards of reporting trials (CONSORT) flow of the entire study is depicted in Figure 1.

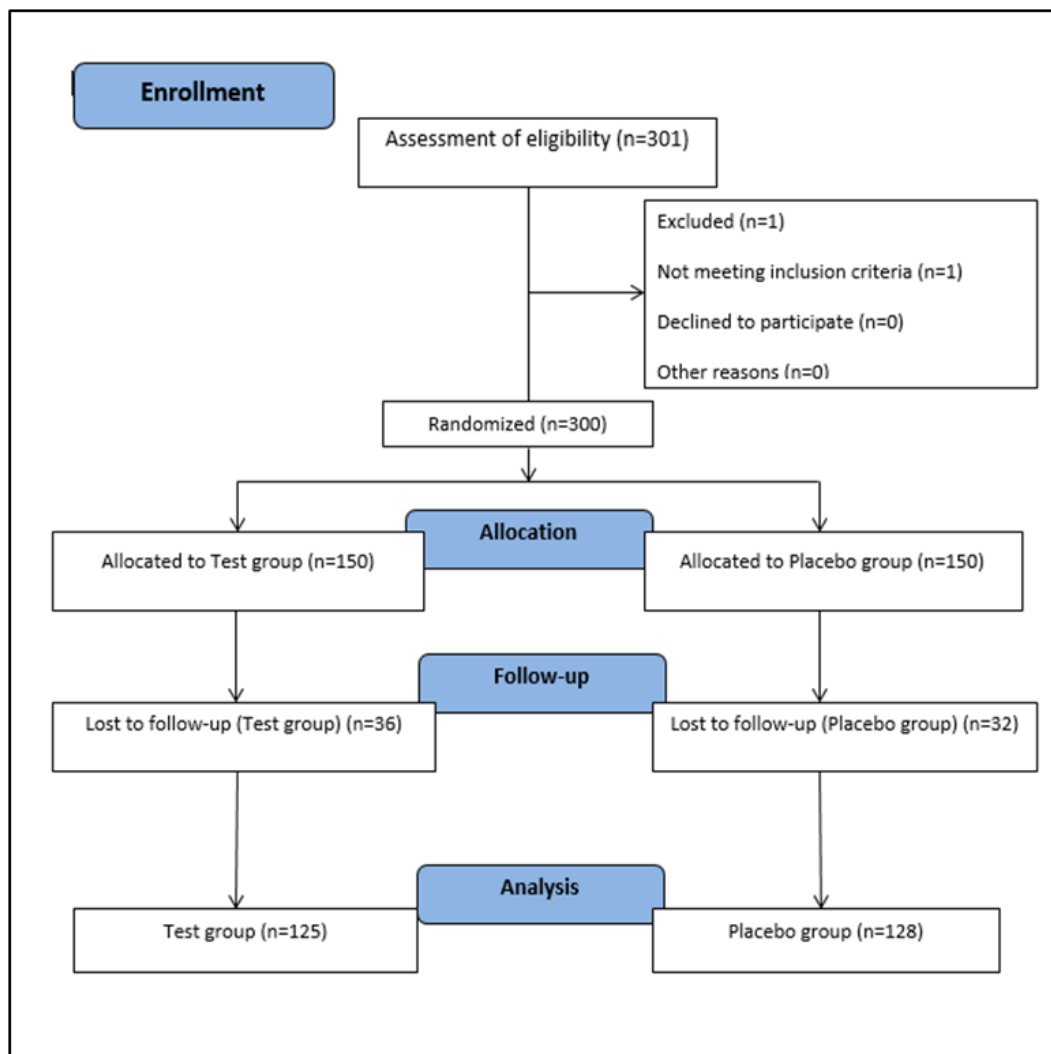


Figure 1: CONSORT flow diagram

Inclusion criteria

Male subjects willing to provide written informed consent to participate in the study and willing to follow up were enrolled in the study. The male subjects aged between 25 –45 years were considered. The male subjects whose female partner was defined to be fertile were considered. The male subjects indicated sperm DNA fragmentation >20% were enrolled.

Exclusion criteria

Subjects with General and endocrinological diseases (clinical examination and routine hormonal laboratory tests) were excluded from the study. The subjects with Previous or present cryptorchidism or genital obstruction were not considered. Subjects with reproductive endocrinological disorders/ genital obstructions were excluded. Subjects having a history of vasectomy, undescended testis, prostate cancer, varicocele, and hydrocele were excluded. Subjects having a history of chemotherapy or radiation for malignant conditions and a history of Azoospermia were excluded.

Study groups

Three hundred subjects were randomized using computer-generated randomization to receive either treatment or placebo.

Sample size

The sample size was calculated by a trained statistician of around 270 subjects hence 300 subjects were enrolled in the study i.e. 150 subjects per group at 90% power and 5% level of significance.

Intervention and dosage

Oligocare Forte Plus is a combination of micronutrients, essential amino acids, antioxidants, and vitamins that are essential for the male reproductive system. The Oligocare Forte Plus tablets contain the following key ingredients Coenzyme Q-10, L-Carnitine, L-Arginine, L-Glutathione, Vitamins like C, E, B6, B12, B1, A, and D Ginseng Extract, Lycopene along with micronutrients like elemental zinc, iron, copper selenium, manganese, folic acid etc.

Therapeutic rationale

Evidence suggests that reactive oxygen species (ROS)-mediated damage to sperm is a significant contributing pathology in 30–80% of cases. ROS, causes infertility by two principal mechanisms. First, damage to the sperm membrane which in turn reduces the sperm's motility and ability to fuse with the oocyte. Secondly, damage to sperm DNA compromises the paternal genomic contribution to the embryo [9]. Considering the antioxidants and micronutrients present in Oligocare Forte Plus and their role in alleviating ROS the present study was proposed to evaluate the efficacy of Oligocare Forte Plus in subfertile males.

Dosage and Administration

Oligocare Forte Plus and placebo tablets were advised to be consumed once daily after the main meal with water for three months. The dose of Oligocare Forte Plus was decided as per various clinical studies and the Recommended Dietary Allowance (RDA) requirement of each ingredient.

Outcome measures

The study outcomes of the present study were to evaluate changes in sperm DFI (DNA Fragmentation Index) in sub-fertile males after treatment from baseline to end of the study along with overall pregnancy incidence amongst the study couple.

Methodology

This was a randomized, multicentric, double-blind, placebo-controlled interventional, prospective, comparative clinical study

conducted in three hundred sub-fertile male subjects across ten study sites in India.

On the screening visit, written informed consent was obtained from the subjects for their participation in the study. Subject's demographics, medical, surgical, treatment history, and current medication if any were recorded. The clinical assessment of the subject was made on each visit together with sperm DFI analysis at baseline and day 90. We have processed all semen samples for the DFI analysis at a centralized laboratory to get uniformity in assessment. Sexual abstinence for 3 days before the semen collection was advised to each participant. The enrollment of subjects was done based on the inclusion/exclusion criteria. We also investigated to rule out all endocrine disorders in all the subjects to avoid any confounder bias. Enrolled subjects were randomized to one of the two study groups (either Oligocare Forte Plus or placebo group) in a 1:1 ratio as per the computer-generated randomization schedule. The investigational products (IPs) were masked to make their appearance identical for both the treatment arms. Subjects were advised to continue their concomitant medication other than antioxidant agents, vitamins, nutraceutical, ayurvedic, or herbal medication. Subjects were advised to continue the diet and exercise regimen during the entire study period. Subjects were called for follow-up visits per month. The pregnancy incidence if any was recorded. Drug compliance was assessed by the investigator on every follow-up visit. All the subjects were offered lifestyle modification counselling such as eating a balanced diet, regular light exercise, and quitting alcohol, smoking, and unhealthy food.

Data analysis

Patients without any major protocol violation were included in the per-protocol population, including those patients who had good treatment compliance, who did not take any prohibited medications during the study period, and whose assessment reports were performed as requested. The safety population consisted of all patients enrolled in the study, who had received at least one dose of study products.

Analysis of efficacy parameters

The mean values of the efficacy parameters were assessed by student t-test and the frequency of the pregnancy incidences was analyzed by Fisher exact test.

Safety Analysis

Adverse events (AEs) and serious adverse events (SAEs) were summarized, counting the number of separate events and the number of subjects experiencing events occurring during the study period.

Results

The mean age for the subjects randomized in treatment and placebo groups were comparable statistically (35 ± 5.1 years and 34 ± 5 years respectively).

Changes in DFI % in population with DFI more than 20%:

The mean DFI% in the Oligocare forte plus group was 32.09 and 30.71 in the placebo group which was comparable at baseline. There was an 18.63% decrease in sperm DFI in the Oligocare forte plus group compared to 12.63 % in the placebo group. Oligocare forte plus treatment led to a statistically significant decrease in total sperm DFI% when compared to baseline (Table 1).

Changes in DFI % in different slabs:

For evaluating the effectiveness of intervention by Oligocare Forte Plus we further stratified the subjects in a population with DFI, 20-30%, 31-40%, and above 40% post hoc. The criteria-wise

distribution of subjects is depicted in Figure 2 and observed to be comparative between Oligocare forte plus and placebo groups at baseline.

In a subset of DFI between 20-30% at baseline, there was no statistical difference in the DFI% in both groups after treatment. In a subset of DFI between 31-40% and above 40% at baseline, there was a significant decrease in DFI in both groups but the percent reduction is greater in the Oligocare Forte Plus group than placebo (Table 2, Figure 3).

Assessment of overall pregnancy incidence amongst the study couple:

There were 25 couples from the Oligocare forte plus treatment group with pregnancy compared to 21 in the placebo group.

There were no adverse events related to study medication or possible engagement of test intervention reported throughout the study period.

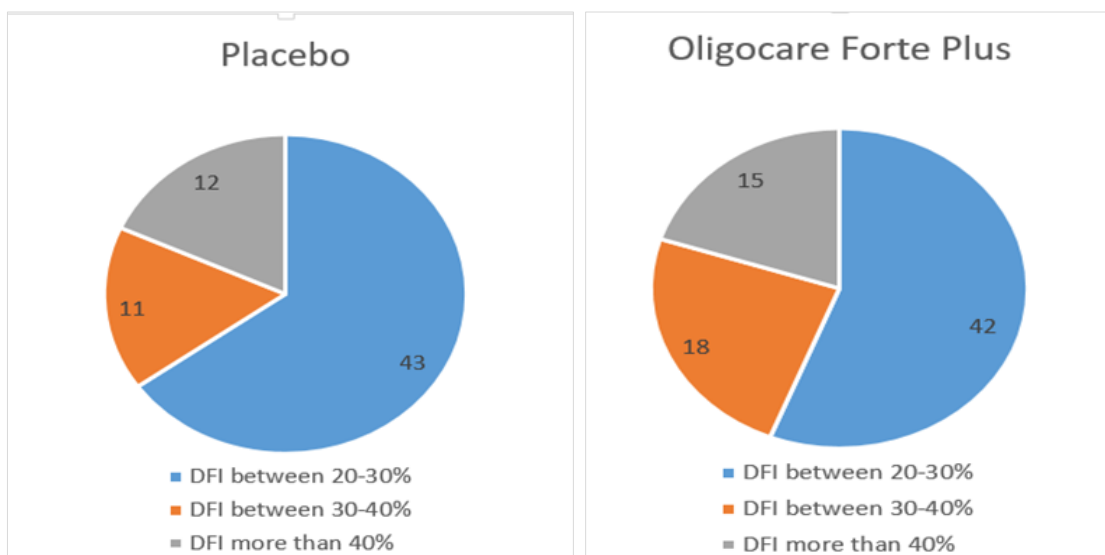


Figure 2: The distribution of subjects according to DFI% slabs

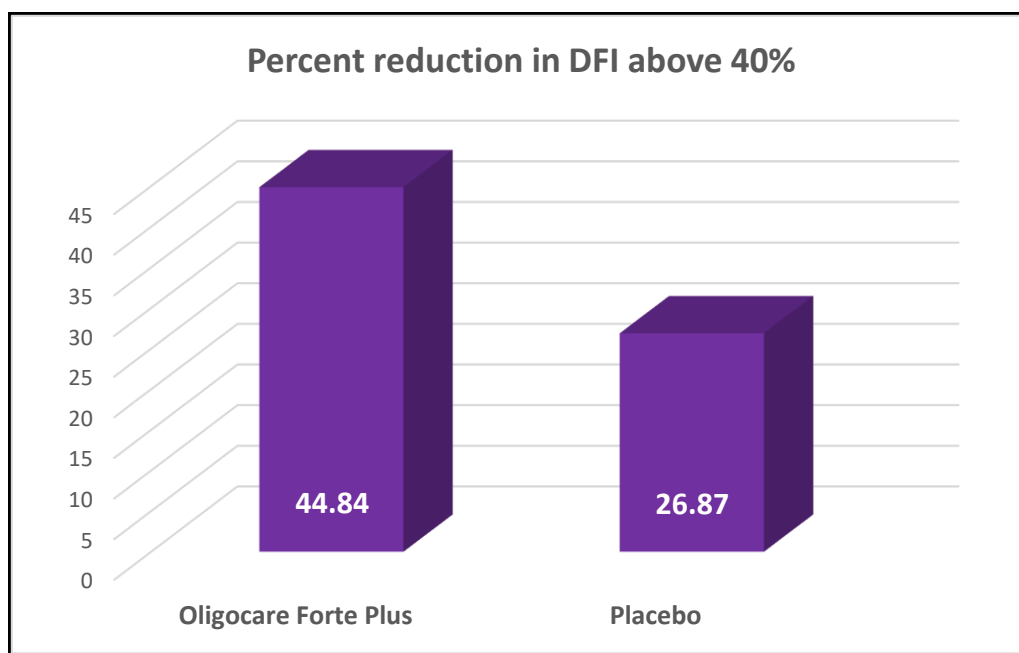


Figure 3: Changes in percent reduction of DFI in population with DFI>40%

Table 1: Changes in percent reduction of DFI in population with DFI>20%

Parameters	Treatment(N=75)		% Decrease	P value	Placebo (N=66)		% decrease	P value
	Screening	Day 90			Screening	Day 90		
DFI	32.09±12.37	26.11±11.63	18.63	0.0051	30.71±10.34	26.83±14.29	12.63	0.0110
Difference	5.99±17.02				3.88±14.06			

Discussion

There is a significant reduction in percent DFI in the Oligocare forte plus treated group than placebo. In a subset analysis, it was found that there was a greater reduction in percent DFI in Oligocare forte plus treated group patients whose baseline DFI is more than 30%.

The pregnancy incidence was relatively higher in the Oligocare forte plus group than placebo (25 Vs. 21).

Throughout the trial, no adverse events or clinical examination abnormalities were documented, providing further evidence that the Oligocare Forte Plus is tolerable and safe for use. Consistent with our findings, research conducted on a global scale

has also established the critical significance of nutrition, vitamins, and minerals in maintaining healthy sperm [8].

In clinical terms, sperm DNA fragmentation is recognized as a substantial factor in infertility. Beyond its association solely with the moment of conception, the correlation between DNA damage and a heightened incidence of spontaneous abortions and compromised embryo quality is adverse [5]. Overall, DNA damage diminishes the reproductive success of males.

The vitamin composition of Oligocare Forte Plus tablets is well-balanced, including A, D, E, C, B12, and B6. Globally, clinical research has demonstrated that these vitamins can reduce DNA fragmentation [9]. Vitamins combined with micronutrients such as selenium, zinc, manganese, copper, and iron have been shown in certain studies to reduce DFI by 15-17% when used as an antioxidant combination [10]. Oligocare Forte Plus Tablets include a combination of minerals and vitamins of superior quality to support male fertility. Oligocare Forte Plus contains L-carnitine, L-arginine, and L-Glutathione, which are vital for energy metabolism and spermatozoa maturation and can preserve sperm DNA integrity [11]. The use of ginseng as a dietary supplement has been shown to decrease the DFI percentage in infertile males [12].

The primary objective of any intervention designed to enhance sperm quality and quantity should be to safeguard sperm against oxidative damage while preserving their normal count and functionality to achieve conception. In our research, therapy with Oligocare Forte plus improved the sperm count, motility, and normal morphology of subfertile males. The data is not presented here.

Conclusion

Nutritional supplementation and lifestyle modifications offer a holistic approach to the management of Oligoasthenoteratozoospermia and hence fertility. Adequate intake of vitamins, and minerals, can improve sperm quality and quantity. Additionally, a balanced diet rich in antioxidants, omega-3 fatty acids, and proteins supports reproductive health. Lifestyle changes, including regular exercise, stress management, avoiding tobacco and excessive alcohol, and maintaining a healthy weight, play crucial roles. Combining nutritional supplementation with lifestyle modifications enhances overall well-being and offers a comprehensive strategy to combat oligospermia, promoting fertility naturally. The current study serves as a clinical validation of the efficacy of Oligocare Forte Plus for treating Oligoasthenoteratozoospermia, aiming to establish its viability as a therapeutic option before considering IUI, ART, or IVF procedures.

Declaration of conflict of interests

Mr. Rohit Shelatkar is a part of Vitabiotics UK. Meyer Organics Pvt. Ltd. is a group company of Vitabiotics. Other authors declare no conflict of interest.

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