Section: Infertility

Original Research Article

EVALUATING MALE FERTILITY OUTCOMES (CAPACITATION, ACROSOME REACTION, PENETRATION TO ZONA PELLUCIDA, MOTILITY, COUNT, MORPHOLOGY AND SEMEN QUALITY) WITH A PATENTED PROPRIETARY FORMULATION CONTAINING D-ASPARTATE, BETAINE, AND UBIQUINOL COMPARED TO COMPARATIVE THERAPY: CAPTURE TRIAL

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ABSTRACT

Background: The male is solely responsible for about 20% and is a contributing factor in another 30% to 40% of all infertility cases. Overall, the male factor is contributory in about 50% of all cases of infertility. The aim of the study is to highlight unaddressed factors contributing to unexplained/idiopathic male infertility and to assess the effectiveness and safety of Oligonorm®, a novel formulation containing D-Aspartic Acid, Ubiquinol, and Betaine, in enhancing male fertility parameters.

Materials and Methods: This was a 16-week randomized, double blind, clinical trial comparing Oligonorm® with a comparative therapy (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc, and Vitamins). A total 100 male participants aged between 25 to 45 years diagnosed with infertility or subfertility, including Asthenospermia, Azoospermia, Oligospermia, or Teratospermia, were enrolled and randomized to either the Oligonorm® (a synergistic blend of D-Aspartic Acid, Ubiquinol acetate, and Betaine) group (n=50) or the comparative group (n=50). The study evaluation includes sperm parameters (count, motility, viability, and capacitation), hormonal status assessment (serum testosterone levels) and adverse event surveillance throughout the study conduct.

Results: Oligonorm® exhibited significant increase in sperm count (baseline: 12.2 million/mL, Week 12: 28.0 million/mL), live sperm percentage (baseline: 37.1%, Week 12: 73.7%) and testosterone (baseline: 332 ng/dL, Week 12: 690

ng/dL) compared to baseline. Oligonorm® group showed significant improvement in semen volume (baseline: 3.1 mL, Week 12: 3.6 mL), sperm motility, sperm-hyaluronic acid binding and acrosome reaction compare to comparative group. Dead sperm percentage declined in Oligonorm® group (baseline: 60.4%, Week 12: 26.3%). It exhibited a favourable safety profile with no notable adverse events. Overall, Oligonorm® significantly improved multiple male fertility parameters, suggesting its potential as a comprehensive solution for male infertility. The synergistic effects of D-Aspartic Acid, Ubiquinol acetate, and Betaine in Oligonorm® contributed to enhanced sperm count, semen volume, motility, viability, sperm capacitation, and testosterone levels.

Conclusion: This study provides robust evidence supporting the efficacy and safety of Oligonorm® in improving male reproductive health. The positive impact on various sperm parameters suggests Oligonorm®'s potential as a promising intervention for male infertility. Further research is warranted to validate these findings and explore the long-term effects of Oligonorm® on male reproductive health.

Keywords: Sperm capacitation, idiopathic male fertility, infertility, male fertility, Oligonorm®, sperm count, sperm motility, unexplained male fertility.

INTRODUCTION

Infertility, affecting 1 in 6 individuals globally, remains a universal reproductive health concern, particularly influenced by male-related factors such as sperm quality and functions.[1,2] Researchers are encouraged to prioritize investigations into unexplained or idiopathic male infertility, a condition characterized by the absence of an immediately apparent cause. This category is critical because it includes cases without obvious physical or genetic abnormalities, yet central spermatogenesis is suppressed, leading to low sperm production. Non-obstructive azoospermia (NOA) represents the most severe form, affecting 10 to 15% of male infertility cases. Unlike obstructive azoospermia, which requires surgical or genetic interventions for physical blockages, NOA is characterized by normal physical and genetic traits but lacks sperm production.[3]

A study published in Fertility and Sterility reported the presence of D-aspartic acid as an endogenous compound in seminal plasma and spermatozoa. They found that the concentration of D-aspartic acid in the seminal plasma of oligoasthenoteratozoospermic (OAT) donors and azoospermic donors is significantly reduced compared to that found in normospermic donors. Similarly, in spermatozoa of OAT donors, the concentration of D-aspartic acid reduced by 50%, which was statistically significant, indicating a substantial decrease in aspartate levels correlating with the severity of male infertility. This suggests that measuring D-aspartic acid could be a valuable tool in assessing the severity of male infertility in unexplained or idiopathic cases.[4]

Similarly, another study reported the presence of single nucleotide polymorphism (SNP) in the choline dehydrogenase (CHDH) gene as a major factor for idiopathic male infertility. Consequences of the CHDH gene polymorphism include altered sperm motility patterns, dysmorphic mitochondrial ultrastructure, high DNA fragmentation index (DFI), and a significant reduction in ATP concentrations in sperm. These effects ultimately lead to sperm de-capacitation, contributing to male infertility. [5]

Further, our research builds upon existing data from research that have shown a direct correlation between D-aspartic acid levels and the severity of male infertility, by measuring D-aspartic acid levels and observing the effects of Oligonorm® (D-aspartic acid, Betaine, and Ubiquinol) D-aspartic acid, an amino acid derivative predominantly found in neuroendocrine tissues has gained attention for its potential role in male fertility enhancement. Previous studies suggest that D-aspartic acid can stimulate the synthesis and secretion of testosterone, a pivotal hormone essential for spermatogenesis and reproductive health. Additionally, D-aspartic acid has been demonstrated to boost sperm quality by augmenting sperm motility and enhancing mitochondrial function within sperm cells, a key constituent of Oligonorm®, which is associated with increased semen volume, sperm count, motility, and viability and we aim to demonstrate its potential in elevating D-aspartic acid levels. The second aspect of our study addresses the issue of sperm decapacitation which contributes to unexplained or idiopathic male infertility. This phenomenon has been associated with SNP polymorphism in the CHDH gene, which inhibits the conversion of choline to betaine, essential for ATP production and ultimately capacitation. Betaine, a metabolite of choline, serves as a crucial ATP source during sperm capacitation. The conversion of choline to betaine occurs in the mitochondrial matrix, facilitated by choline dehydrogenase (CHDH), which transports choline across mitochondrial membranes. Single nucleotide polymorphisms (SNPs) in the CHDH gene have been associated with betaine deficiency, leading to dysmorphic mitochondrial structure, altered sperm motility patterns, decreased energy levels in sperm, and increased osmotic pressure.[5] The second component of Oligonorm® also contains Betaine, which helps to overcome deficits in CHDH function, thereby potentially improving male fertility outcomes. Ubiquinol acts by

reducing reactive oxygen species (ROS) and preventing sperm apoptosis, thereby enhancing sperm count and motility, we aim to sidestep this polymorphism and enhance ATP production, potentially improving sperm motility and capacitation.[6,7]

In our study, we have addressed the complex issue of unexplained or idiopathic male infertility by focusing on two key aspects. Firstly, we aim to investigate the role of D-aspartic acid deficiency in seminal plasma, examining its association with hypo-spermatogenesis and idiopathic male fertility. Secondly, we are exploring the impact of a single nucleotide polymorphism (SNP) in the choline dehydrogenase (CHDH) gene, which affects the conversion of choline to betaine, a crucial process for ATP production in sperm. Overall, our study highlights unaddressed factors contributing to unexplained/idiopathic male infertility and evaluates the potential of D-Aspartic Acid, Betaine, and Ubiquinol acetate (Oligonorm®) as a promising solution for individuals facing such challenges. Therefore, the current study aims to evaluate the effectiveness and safety of Oligonorm® in enhancing male fertility.

MATERIAL AND METHODS

Study Design: This investigation adopts a rigorous 16-week, double-blind, randomized trial design to explore the effects of Oligonorm® in comparison to a comparative therapy (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc, and Vitamins) among male participants aged between 25 to 45 years (both inclusive) diagnosed with infertility or subfertility. Conditions considered for inclusion are Asthenospermia, Azoospermia, Oligospermia, or Teratospermia. A sample size of 100 participants undergoes computer-generated randomization to either the Oligonorm® or the comparative group in a double-blind manner, minimizing bias in outcome assessments.

Study Eligibility

Male participants aged between 25 to 45 years and medically diagnosed with infertility or subfertility (Presence of Asthenospermia, Azoospermia, Oligospermia, or Teratospermia) ready to provide consent, adhere to the study procedures and treatment regimen were enrolled in the study. Another inclusion parameter was a stable sexual relationship for at least one year without contraception, with overlapping conditions, and ready to avoid using other male fertility treatments during the study. Patients were not included those were reported with Systemic or chronic diseases affecting fertility. Having a history of urogenital abnormalities or untreated varicocele is on recent use (past six months) of medications affecting fertility, Genetic abnormalities or chromosomal disorders affecting fertility, active alcohol or substance abuse, recent participation in other clinical trials involving investigational drugs or devices. Patients with severe psychiatric or psychological disorders, Current or planned participation in fertility treatment procedures during the study. Any condition interfering with participant safety or study integrity.

Duration of Treatment: The study comprises a 16-week timeline, including a 2-week Screening Phase (Week -2 to Week 0), a 12-week Treatment Phase, (Visits 3 to 6), (Day 14 / Week 2 to Day 85 / Week 12), and a 2-week Follow-up Phase (Week 14).

Assessments and Procedures: The research employs a comprehensive array of evaluations and methodologies, including the analysis of sperm parameters (count, motility, viability, and capacitation) and hormonal status assessment (serum testosterone levels). Adverse event surveillance meticulously documents and evaluates participant experiences, while compliance evaluation gauges adherence to the prescribed treatment regimen.

Methodology of Semen Analysis

Participants meeting inclusion criteria were instructed to abstain from ejaculation for 2-5 days. Semen samples were collected using sterile containers. Semen samples were allowed to liquefy at room temperature for 20-30 minutes. It was measured using a calibrated graduated cylinder, with recorded volume reported in millilitres (ml). Sperm parameters as sperm count was determined using a hemocytometer or automated sperm counting device. Dilution was performed, when necessary, with counts obtained from multiple fields. sperm mobility (Motility) was assessed under a microscope, and categorized into progressive, non-progressive, and immotile, adhering to established criteria. Sperm viability was determined using a vital stain (e.g., eosin-nigrosin) to distinguish live and dead sperm. Counts of stained and unstained sperm were recorded. Sperm capacitation was assessed using two specific methods: Sperm bound to hyaluronic acid (HA) and sperm undergoing acrosome reaction.

Statistical Analysis

The data acquired from the investigation were subjected to analysis using suitable statistical techniques, such as t-tests, ANOVA, and regression analysis, to evaluate and compare the results between the Oligonorm® and comparative cohorts, with the aim of identifying any statistically significant disparities.

RESULTS

Demographic Distribution

A total of 100 male participants were enrolled in the study and randomized into two groups: the Oligonorm® group (n=50) and the comparative group (n=50). The mean age of the participants was 30±1 years.

Within the Oligonorm® Group, the mean age of the enlisted subjects was 31 years. Out of the total sample size of 50 participants, the study identified 11 individuals with Asthenoteratospermia, a condition characterized by both asthenozoospermia and

teratozoospermia. Additionally, 11 participants were diagnosed with Oligospermia, indicating a lower-than-normal sperm count. Notably, Asthenoteratospermia and Oligospermia emerged as the diagnostic categories with the highest number of recruits within the Oligonorm® Group. The distribution of participants across other diagnostic categories includes 1 participant with Asthenospermia, 6 with Azoospermia, 6 with Teratozoospermia, 8 with Asthenozoospermic conditions, and 7 with teratozoospermia with oligospermia.

In the comparative group (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc, and Vitamins) the average age of participants stood at 30 years. Notably, the Asthenoteratospermia category exhibited the highest recruitment with 12 participants, suggesting a significant representation of individuals with both asthenozoospermia and teratozoospermia within this subgroup. Additionally, the group showed a diagnosis of teratozoospermia with oligospermia in 11 participants, underscoring the coexistence within this specific subset. Further breakdown of diagnostic categories revealed that the group comprised 9 participants with Asthenozoospermia, 3 with Azoospermia, 7 with Oligospermia, and 8 with Teratozoospermia.

Semen Volume

In the Oligonorm® group, participants exhibited an increase in semen volume from a baseline value of 3.1 mL to 3.6 mL at Week 12. On the other hand, the comparative group (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) showed a modest increase, with semen volume (refer Figure 1) changing from 3.1 mL at baseline to 3.2 mL by Week 12. These results suggest that both groups experienced an improvement in semen volume, despite a slightly more pronounced increase in the Oligonorm® Group. The findings imply that the Oligonorm® intervention might have contributed to a more substantial enhancement in semen volume compared to the comparative group combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins). However, it's worth noting that the differences in semen volume changes between the two groups were relatively minor, and further analysis may be required to determine the clinical significance of these changes.

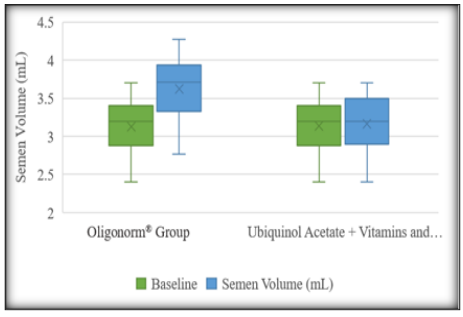

Figure 1: Comparison of Mean Change in Semen Volume between Oligonorm® Group and comparative Group between Baseline and Week 12.

Sperm Count

The result indicates a notable increase in sperm count (measured in millions per millilitre, million/mL) over the course of the study. In the Oligonorm® group, participants exhibited a substantial rise from a baseline sperm count (refer figure 2) of 12.2 million/mL to 28.0 million/mL at Week 12. Similarly, in the comparative combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) Group, the sperm count increased from 13.6 million/mL at baseline to 23.8 million/mL by Week 12. These findings demonstrate that both the Oligonorm® Group and the comparative combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) Group experienced an improvement in sperm count during the study period. While the Oligonorm® Group showed a more significant increase, both groups displayed a noteworthy enhancement in sperm count, which suggests the potential efficacy of the interventions in positively affecting sperm production.

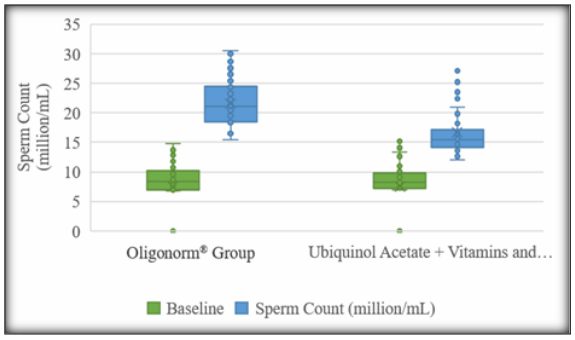

Figure 2: Comparison of Semen Volume Changes Over 12 Weeks in Oligonorm® and comparative combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) Group.

Sperm Motility

For the Oligonorm® group, at baseline, the mean sperm motility was 14.3% for progressive motility (PR), 27.8% for non-progressive motility (NP), and 29.9% for immotility (IM). After 12 Weeks, these values significantly improved to 63.7% for PR, 18.5% for NP, and 17.8% for IM. Comparatively, within the other (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group, at baseline, the mean sperm motility was 13.6% for PR, 25.14% for NP, and 37.26% for IM. Following 12 Weeks, these values changed to 23.62% for PR, 30.57% for NP, and 45.82% for IM.

Overall, both groups experienced improvements in sperm motility parameters during the study. In the Oligonorm® group, the improvements were particularly significant, as demonstrated by the substantial increase in PR, decreased NP motility percentages, and decreased IM. In the comparative combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group, improvements were also observed, although to a lesser extent. The data suggests that the Oligonorm®

intervention had a more pronounced positive impact on sperm motility, both in terms of progressive and non-progressive motility, compared to the (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group. (Refer to figure 3a, 3b and 3c.)

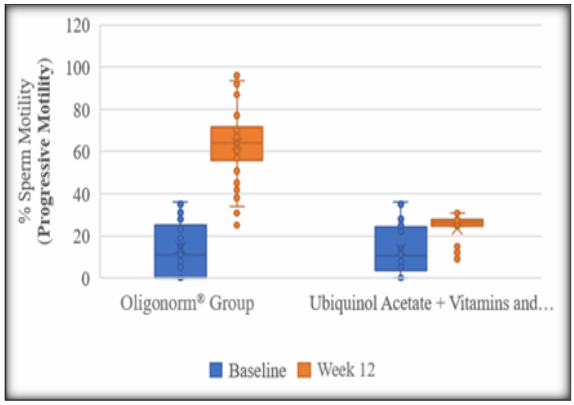

Figure 3a: Comparison of Sperm Motility (Progressive Motility) Changes Over 12 Weeks in Oligonorm® and comparative Combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group.

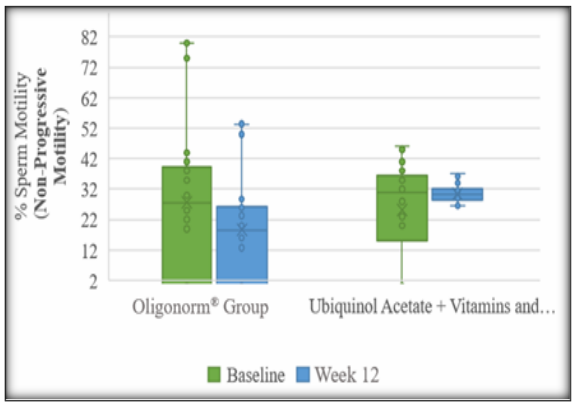

Figure 3b: Comparison of Sperm Motility (Non-Progressive Motility) Changes Over 12 Weeks in Oligonorm® and Comparative Combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group.

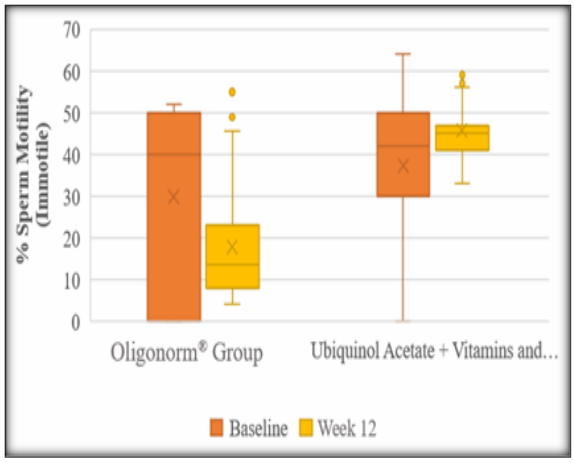

Figure 3c: Comparison of Sperm Motility (Immotile) Changes over 12 Weeks in Oligonorm® and combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group

Sperm Viability

In the Oligonorm® group, the percentage of live sperm significantly rose from 37.1% to 73.7% by Week 12, indicating a significant enhancement in sperm viability. Concurrently, the percentage of dead sperm notably declined from 60.4% to 26.3%, demonstrating a substantial reduction in sperm cell mortality. In contrast, the comparative combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group witnessed a moderate increase in live sperm from 37.1% to 49.9% by Week 12, accompanied by a rise in dead sperm from 56.6% to 50.1% as shown in figure 4a and b. These results accentuate Oligonorm®'s positive impact on sperm viability, highlighting significant increases in live sperm and decreases in dead sperm percentages. Notably, the Oligonorm® Group displayed more pronounced improvements compared to the other (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group.

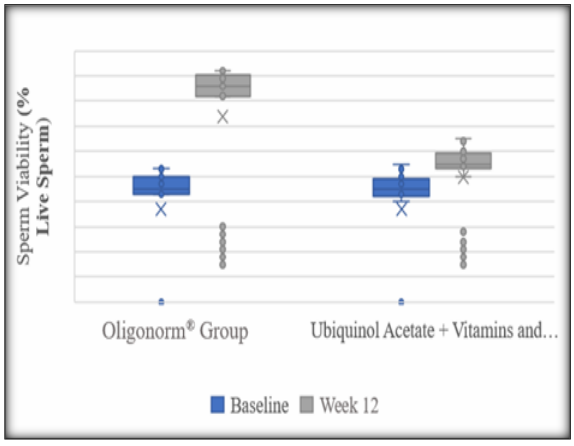

Figure 4a: Comparison of Sperm Viability (% Live Sperm) Changes Over 12 Weeks in Oligonorm® and Comparative Combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) Group.

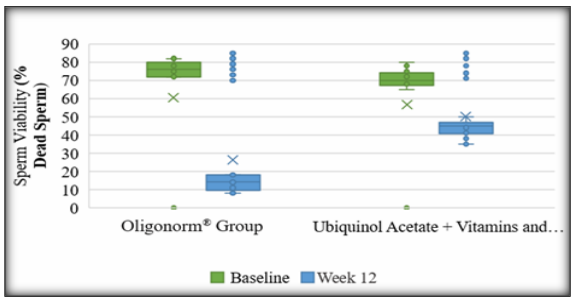

Figure 4b: Comparison of Sperm Viability (% Dead Sperm) Changes Over 12 Weeks in Oligonorm® and comparative Combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) groups

Sperm Capacitation

In the Oligonorm® Group, the baseline mean percentage of sperm bound to Hyaluronic Acid (HA) was 35.0%, experiencing a remarkable increase to 71.5% by Week 12. This notable augmentation signifies an amplified capability of sperm to engage

with HA, a pivotal component in the process of sperm-egg binding during fertilization. Concurrently, the mean percentage of sperm undergoing the acrosome reaction rose from 29.5% to 52.6% by Week 12, indicating an advancement in sperm maturity and an enhanced potential for successful fertilization events as briefed in Figure 5a.

Conversely, within the comparative (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group, the observed changes were more subdued. The mean percentage of sperm bound to HA displays a relatively stable transition, shifting from 35.7% at baseline to 53.1% by Week 12. Similarly, the mean percentage of sperm undergoing the acrosome reaction exhibited a slight rise, progressing from 29.6% to 39.3% over the corresponding period as per Figure 5b. These findings suggest that while the comparative combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group experienced improvements, the Oligonorm® group demonstrated more substantial enhancements in terms of sperm-HA binding capability and the progression of sperm towards fertilization competence.

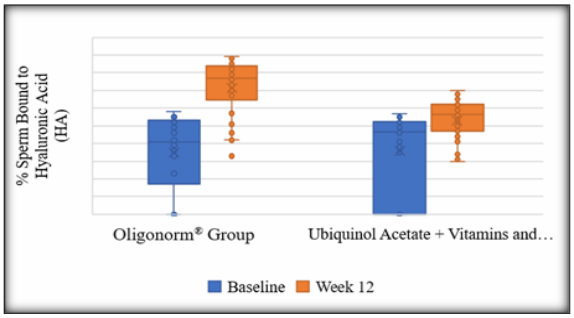

Figure 5a: Comparison of % Sperm Bound to Hyaluronic Acid (HA) Changes Over 12 Weeks in Oligonorm® and Comparative Combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) Group

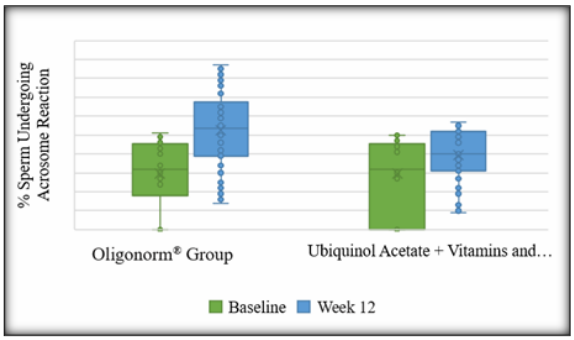

Figure 5b: Comparison of % Sperm Undergoing Acrosome Reaction Changes Over 12 Weeks in Oligonorm® and comparative combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group

Testosterone Levels

In the Oligonorm® group, baseline testosterone levels increased substantially from 332 ng/dL to 690 ng/dL by Week 12, highlighting the potential of Oligonorm® to enhance testosterone secretion crucial for male reproductive health. In contrast, the comparative combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group showed more modest changes, with baseline levels of 339 ng/dL decreasing to 468 ng/dL by Week 12 as mentioned in figure 6. These findings suggest Oligonorm®'s potential positive influence on hormonal balance, underlining its role in male reproductive health enhancement.

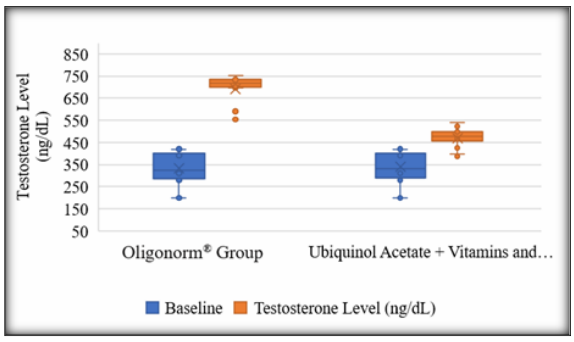

Figure 6: Comparison of Testosterone Level Changes Over 12 Weeks in Oligonorm® and comparative Combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) Group

D-Aspartic Acid Level

In the Oligonorm® Group, baseline testosterone levels increased from 13 ng/dL to 24 ng/dL by Week 12, indicating a significant rise. Meanwhile, the comparative combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group experienced a more modest change, with baseline levels of 12 ng/dL increasing to 14 ng/dL by Week 12 (refer Figure 7). These findings suggest both interventions influenced testosterone levels, with Oligonorm® showing a more pronounced impact.

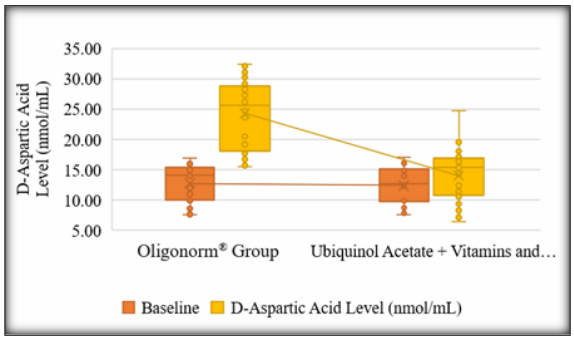

Figure 7: Comparison of D-Aspartic Acid Changes Over 12 Weeks in Oligonorm® and comparative combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) Group

DNA Fragmentation Index

In this comparative analysis of the Oligonorm® Group and the comparative combination, group (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) significant

differences are observed at baseline and Week 12. The Oligonorm® group demonstrated a significant decrease in average values in DFI from 30.17% at the beginning of the study to 12.48% at Week 12, along with a decrease in the middle value from 32.9% to 13.9%. This observation suggests a significant change in the mean or average of the data collected by the Oligonorm® group. Furthermore, the observed standard deviation exhibited a decrease from 12.25% to 2.97%, indicating a notable reduction in variability. In contrast, the group administered with the comparative group (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) exhibited a decrease in DFI average values from 32.40% at the beginning of the study to 22.82% at Week 12. Additionally, there was a reduction in the middlemost values from 33.4% to 23.2%. The observed standard deviation within this group exhibited a decrease from 9.75% to 7.78% (refer figure 8). The observed results suggest notable changes in both cohorts throughout the duration of the study, indicating the need for additional examination and analysis.

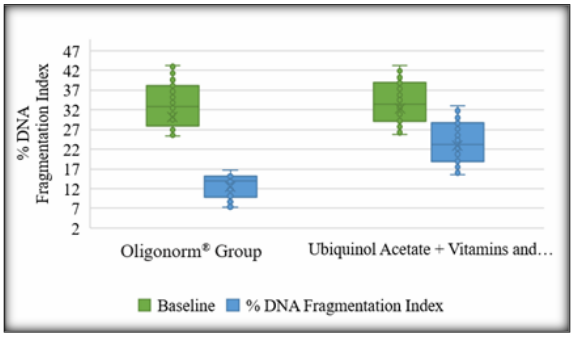

Figure 8: Comparison of % DNA Fragmentation Changes Over 12 Weeks in Oligonorm® and comparative combination (Ubiquinol Acetate, L-Carnitine, Lycopene, Astaxanthin, Zinc and Vitamins) group

DISCUSSION

The results of this randomized, double-blind, clinical investigation show that Oligonorm® is effective at increasing male reproductive indices. The results show that compared to the comparative group, those using Oligonorm® experienced significant increases in sperm count, semen volume, sperm motility, viability, sperm capacitation, and testosterone. The observations presented in this study are consistent with the established scientific evidence regarding the individual impacts of the key components found in Oligonorm®. This provides a strong foundation for considering its potential as a supplement that enhances male fertility.

The observed elevation in sperm count within the Oligonorm® group aligns with prior scientific investigations regarding the influence of D-Aspartic Acid on male reproductive well-being.[5] D-Aspartic Acid, a key constituent of Oligonorm®, has been documented to exhibit the ability to enhance the synthesis of luteinizing hormone (LH) and testosterone.[5,8,9] These hormones are essential for the regulation of spermatogenesis and the maturation of sperm, ultimately impacting sperm count and male fertility as a whole.[4] The findings of the present study indicate that the concurrent administration of D-Aspartic Acid, Betaine and Ubiquinol acetatein Oligonorm® may enhance its impact on sperm count, resulting in a significant enhancement in sperm production with potential clinical relevance.

The observed increase in semen volume in the Oligonorm® group may be attributed to the synergistic effects of D-Aspartic Acid and Betaine. Previous studies have demonstrated that D-Aspartic Acid has the potential to increase the volume of seminal fluid.[4,9] Additionally, Betaine has been linked to enhanced sperm energy and viability. This combination has the potential to exhibit synergistic effects that may enhance the process of spermatogenesis and contribute to the maintenance of overall reproductive well-being.

The observed increase in sperm motility, encompassing both overall motility and rapid motility, in the Oligonorm® experimental group aligns with previous research findings regarding the impact of D-Aspartic Acid and Ubiquinol acetate on sperm functionality.[5,10,11] Studies have shown that D-Aspartic Acid has the ability to improve the movement of sperm, known as sperm motility. On the other hand, Ubiquinol acetate functions by reducing the levels of reactive oxygen species (ROS),[12,13] which are known to have detrimental effects.[14] Elevated levels of reactive oxygen species (ROS) have the potential to induce oxidative harm to the deoxyribonucleic acid (DNA) of sperm cells,[15,16] thereby compromising their functionality and ultimately playing a role in the development of infertility. Through the mitigation of reactive oxygen species (ROS) and the inhibition of sperm apoptosis, the presence of Ubiquinol acetate in Oligonorm® has the potential to enhance the quality and motility of sperm, thereby providing support for the existing research outcomes.[17]

Moreover, the observed increase in sperm capacitation in the Oligonorm® group indicates a potential involvement of Betaine in facilitating sperm maturation and enhancing fertilization ability. Capacitation is an essential process in the progression of sperm toward successful conception, and previous studies have indicated that Betaine can enhance sperm vitality and capacity for egg fertilization.[18,19] The findings of this investigation, thus, emphasize the potential of Oligonorm® to augment sperm capacitation and enhance fertilisation efficacy.

The observed elevation in testosterone concentrations within the Oligonorm® cohort aligns with prior scientific investigations that have established the androgenic characteristics of D-Aspartic Acid. Testosterone is an essential factor in the process of spermatogenesis, as well as in the regulation of sperm motility and the maintenance of

sperm quality.[20] The observed increase in testosterone levels in the Oligonorm® group may potentially contribute to the overall enhancement of sperm parameters, thus providing further support for the potential advantages of Oligonorm® in improving male reproductive health.[20,21]

Oligonorm®'s safety profile, marked by the absence of notable adverse events, aligns with established research on D-Aspartic Acid,[22,23] Ubiquinol acetate,[24] and Betaine.[25] This reinforces its potential as a safe for improving male fertility. Notably, the comparative group exhibited common side effects like stomach discomfort, bloating, and diarrhea, along with insomnia and itching. In contrast, the Oligonorm® group showed no significant adverse events, emphasizing its favorable safety profile.

In summary, the findings of this investigation offer strong empirical support for the effectiveness of Oligonorm® in enhancing male fertility indicators. The concurrent administration of D-Aspartic Acid, Ubiquinol acetate, and Betaine in Oligonorm® seems to exhibit a synergistic effect in augmenting multiple sperm parameters, testosterone concentrations, and sperm capacitation. The extensive enhancement in various facets of sperm health highlights the potential of Oligonorm® as a superior therapy in the field of male fertility augmentation.

Although the present study provides valuable insights into the effectiveness and safety of Oligonorm®, additional research is necessary to validate and build upon these results. Conducting longitudinal studies to assess the enduring impacts of Oligonorm®, alongside inquiries into its potential drug interactions and effects on various health conditions, would yield significant scientific knowledge regarding its safety and effectiveness. Furthermore, investigating the influence of Oligonorm® on various facets of male reproductive health, such as the integrity of sperm DNA and epigenetic modifications, may provide a more holistic comprehension of its modes of operation and potential uses.

Given the widespread prevalence of male infertility and its significant impact on individuals and couples globally, the distinctive composition and potential advantages of Oligonorm® present a promising prospect for addressing this urgent matter in the field of global health. The multifaceted strategy employed by Oligonorm®, which addresses various aspects of male fertility, has the potential to enhance the likelihood of successful conception and fulfil the aspirations of parenthood for numerous couples experiencing fertility challenges. This, in turn, may contribute to an improved emotional state and overall quality of life for these individuals.

CONCLUSION

The findings from this comprehensive study demonstrate that Oligonorm®, a novel formulation containing D-Aspartic Acid, Betaine, and Ubiquinol acetate, significantly enhances male fertility parameters. The positive impact of Oligonorm® on sperm count, semen volume, sperm motility, viability, sperm capacitation, and testosterone levels provides robust evidence of its efficacy in improving male reproductive health in idiopathic and unexplained conditions.

The synergistic effects of the key ingredients in Oligonorm® appear to contribute to the comprehensive improvement in multiple sperm parameters, positioning it as a superior therapy in the field of male fertility enhancement. Furthermore, the safety and tolerability of Oligonorm®, as evidenced by the absence of significant adverse events, add to its appeal as a potential male fertility enhancer.

These encouraging findings open avenues for additional research and clinical uses of Oligonorm® in treating male infertility. The potential advantages of Oligonorm® in enhancing male reproductive health could greatly benefit numerous couples grappling with fertility issues, potentially providing a hopeful solution to this significant global health concern.

Ethical Statements

The study received ethical approval from the Ethics Committee on 15 Nov 2022. The approval covered all aspects of the research protocol, including participant recruitment, data collection, and analysis procedures.

Statement of Informed Consent

Freely given Informed consent was obtained from all participants involved in this study. Subjects were provided with detailed information regarding the study objectives, procedures, potential risks, and benefits, and their voluntary consent was obtained before inclusion in the study.

Financial Interest Statement

The authors declare no financial interests or conflicts of interest that could influence the research findings. Any potential conflicts have been disclosed and managed in accordance with the International Journal of Medicine and Public Health policies and ethical standards.

REFERENCES

1. WHO. 1 in 6 people globally affected by infertility: WHO.
2. Bach PV, Schlegel PN. Male Infertility. In: Yen and Jaffe's Reproductive Endocrinology. Elsevier; 2019:582-593.e2. doi:10.1016/B978-0-323-47912-7.00023-8
3. Chiba K, Enatsu N, Fujisawa M. Management of non-obstructive azoospermia. Reprod Med Biol. 2016 Jan 18; 15(3):165-173. doi: 10.1007/s12522-016-0234-z
4. D'Aniello, G., Ronsini, S., Guida, F., Spinelli, P., & D'Aniello, A. (2005). Occurrence of D-aspartic acid in human seminal plasma and spermatozoa: possible role in reproduction. Fertility and sterility, 84(5), 1444–1449. https://doi.org/10.1016/j.fertnstert.2005.05.019
5. Johnson AR, Lao S, Wang T, Galanko JA, Zeisel SH. Choline dehydrogenase polymorphism rs12676 is a functional variation and is associated with changes in human sperm cell function. PLoS One. 2012; 7(4):e36047. doi: 10.1371/journal.pone.0036047. Epub 2012 Apr 27.
6. Lee I. (2015). Betaine is a positive regulator of mitochondrial respiration. Biochemical and biophysical research

communications, 456(2), 621–625. https://doi.org/10.1016/j.bbrc.2014.12.005
7. Mohammad Reza Safarinejad, Shiva Safarinejad, Nayyer Shafiei, Saba Safarinejad, Effects of the Reduced Form of Coenzyme Q10 (Ubiquinol) on Semen Parameters in Men with Idiopathic Infertility: a Double-Blind, Placebo Controlled, Randomized Study, The Journal of Urology, Volume 188, Issue 2, 2012, Pages 526-531, ISSN 0022-5347, https://doi.org/10.1016/j.juro.2012.03.131.
8. Macchia G, Topo E, Mangano N, D'Aniello E, Boni R. DL-Aspartic acid administration improves semen quality in rabbit bucks. Anim Reprod Sci. 2010; 118(2-4):337-343. doi:10.1016/J.ANIREPROSCI.2009.07.009
9. Topo E, Soricelli A, D'Aniello A, Ronsini S, D'Aniello G. The role and molecular mechanism of D-aspartic acid in the release and synthesis of LH and testosterone in humans and rats. Reprod Biol Endocrinol. 2009; 7:120. doi:10.1186/1477-7827-7-120
10. Thakur AS, Littattarru GP, Funahashi I, Painkara US, Dange NS, Chauhan P. Effect of Ubiquinol Therapy on Sperm Parameters and Serum Testosterone Levels in Oligoasthenozoospermic Infertile Men. J Clin Diagn Res. 2015; 9(9):BC01. doi:10.7860/JCDR/2015/13617.6424
11. Di Fiore MM, Boni R, Santillo A, et al. D-aspartic acid in vertebrate reproduction: Animal models and experimental designs. Biomolecules. 2019; 9(9). doi:10.3390/biom9090445
12. Ickowicz D, Finkelstein M, Breitbart H. Mechanism of sperm capacitation and the acrosome reaction: role of protein kinases. Asian J Androl. 2012; 14(6):816. doi:10.1038/AJA.2012.81
13. Ochsendorf FR. Infections in the male genital tract and reactive oxygen species. Hum Reprod Update. 1999; 5(5):399-420. doi:10.1093/HUMUPD/5.5.399
14. Bisht S, Faiq M, Tolahunase M, Dada R. Oxidative stress and male infertility. Nat Rev Urol. 2017; 14(8):470-485. doi:10.1038/NRUROL.2017.69
15. Gualtieri R, Kalthur G, Barbato V, et al. Sperm Oxidative Stress during In Vitro Manipulation and Its Effects on Sperm Function and Embryo Development. Antioxidants. 2021; 10(7). doi:10.3390/ANTIOX10071025
16. Aitken RJ, Baker MA. The Role of Genetics and Oxidative Stress in the Etiology of Male Infertility—A Unifying Hypothesis? Front Endocrinol (Lausanne). 2020; 11. doi:10.3389/FENDO.2020.581838
17. Shekarriz M, Thomas AJ, Agarwal A. Incidence and level of seminal reactive oxygen species in normal men. Urology. 1995; 45(1):103-107. doi:10.1016/S0090-4295(95)97088-6
18. Ostermeier GC, Cardona C, Moody MA, et al. Timing of sperm capacitation varies reproducibly among men. Mol Reprod Dev. 2018; 85(5):387-396. doi:10.1002/mrd.22972
19. Puga Molina LC, Luque GM, Balestrini PA, Marín-Briggiler CI, Romarowski A, Buffone MG. Molecular Basis of Human Sperm Capacitation. Front Cell Dev Biol. 2018;6. doi:10.3389/fcell.2018.00072
20. Smith LB, Walker WH. The regulation of spermatogenesis by androgens. Semin Cell Dev Biol. 2014; 30:2-13. doi:10.1016/j.semcdb.2014.02.012
21. Grande G, Barrachina F, Soler-Ventura A, et al. The Role of Testosterone in Spermatogenesis: Lessons from Proteome Profiling of Human Spermatozoa in Testosterone Deficiency. Front Endocrinol (Lausanne). 2022; 13. doi:10.3389/fendo.2022.852661
22. Roshanzamir F, Safavi SM. The putative effects of D-Aspartic acid on blood testosterone levels: A systematic review. Int J Reprod Biomed. 2017; 15(1):1-10.
23. D-Aspartic Acid: Does It Boost Testosterone? Healthline. Accessed August 2, 2023. https://www.healthline.com/nutrition/d-aspartic-acid-and-testosterone
24. Hosoe K, Kitano M, Kishida H, Kubo H, Fujii K, Kitahara M. Study on safety and bioavailability of ubiquinol (Kaneka QHTM) after single and 4-week multiple oral administration to healthy volunteers. Regulatory Toxicology and Pharmacology. 2007; 47(1):19-28. doi:10.1016/j.yrtph.2006.07.001
25. Betaine. Mount Sinai. Accessed August 2, 2023. https://www.mountsinai.org/health-library/supplement/betaine#:~:text=Most%20side%20effects%20from%20betaine,without%20talking%20to%20their%20provider.