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# Effect of Antioxidants on Semen Parameters on SOAT Pt: An Observational Study in Rural Population

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	Abstract		
	This article aims to investigate the effect of antioxidants on semen parameters in the rural population. Male infertility is a growing concern worldwide, and there is evidence emphasizing oxidative stress as a significant contributing factor. Antioxidants have gained attention for their potential role in mitigating the damaging effects of oxidative stress on semen health. This observational study carefully evaluates the impact of antioxidant supplementation on semen parameters in a rural population, contributing to the existing literature on male reproductive health.		
CC License CC-BY-NC-SA 4.0	Key words: Antioxidants, Semen Parameters: Oligo, Astheno, Terato zoospermia, Semen analysis		

#### 1.Introduction

In recent years, there has been a growing concern about the decline in semen quality and male fertility worldwide. Several factors have been linked to this decline, including lifestyle choices, environmental pollution, and oxidative stress. Antioxidants have gained considerable attention as a potential solution to improve semen parameters and protect male reproductive health. This essay aims to explore the effect of antioxidants on semen parameters in a rural population, based on an observational study conducted on SOAT (Semen Oxidation Analysis Test) on oligo astheno terato zoospermia participants.

Semen quality is a crucial factor in male fertility, and its deterioration has become a growing concern in recent years. Several environmental and lifestyle factors, including oxidative stress, are implicated in this decline. Antioxidants have emerged as potential protective agents against oxidative damage, and their role in improving semen parameters is gaining increasing attention.

This observational study aims to investigate the effect of dietary antioxidants on semen parameters (oligo,Astheno, Terato zoospermia) in the rural population of Kajipalasiya, Madhya Pradesh, India. The rural setting provides a unique opportunity to assess the impact of antioxidants in a population with relatively less exposure to industrial pollutants and processed foods, commonly associated with oxidative stress. Specifically, this study will address the following objectives:

• To assess the prevalence of low semen quality among men in Kajipalasiya.

- To evaluate the dietary intake of antioxidants in the study population.
- To investigate the association between dietary antioxidant intake and semen parameters like sperm concentration, motility, morphology, and DNA integrity.
- To explore the potential mechanisms through which antioxidants might influence semen quality.

The rationale for this study is based on the following:

- Oxidative stress: Reactive oxygen species (ROS) generated in the male reproductive system can damage sperm DNA and membranes, leading to reduced sperm quality and fertility.
- Antioxidants: Dietary antioxidants like vitamins C and E, beta-carotene, and selenium can scavenge ROS and protect sperm from oxidative damage.
- Limited research: Despite growing interest in antioxidants and semen quality, research in rural populations, particularly in India, is scarce.

This study, therefore, aims to contribute to the existing knowledge by providing valuable insights into the potential benefits of dietary antioxidants for improving semen quality and male fertility in a rural Indian setting.

The introduction should also include:

- A brief overview of the literature on antioxidants and semen quality.
- The significance of the study for public health and future research.
- A clear statement of the research questions or hypotheses to be addressed.

#### 1.1 Rationale:-

Male infertility affects approximately 15% of couples worldwide, with oxidative stress contributing significantly to a substantial portion of these cases. Sperm cells are particularly vulnerable to reactive oxygen species (ROS) due to their high polyunsaturated fatty acid content and limited antioxidant defenses. This vulnerability can lead to DNA damage, lipid peroxidation, and impaired sperm motility and morphology, all of which negatively impact fertility.

#### 1.2 Semen Oxidative Antistress Test (SOAT):

The SOAT is a diagnostic test that assesses the balance between ROS and antioxidant defenses in the semen. Patients diagnosed with SOAT positivity exhibit elevated levels of oxidative stress, placing them at higher risk for infertility.

#### 1.3 Antioxidants and Fertility:

Antioxidants are molecules that scavenge free radicals and ROS, thus protecting cells from oxidative damage. Supplementation with exogenous antioxidants has been shown to improve sperm quality in men with SOAT positivity, potentially enhancing fertility outcomes. However, the evidence from rural populations remains limited.

#### 1.4 Rural context:

Rural populations often face unique challenges related to access to healthcare and nutritional deficiencies, which can exacerbate oxidative stress and male infertility. Studying the effect of antioxidant supplementation in this context is crucial to developing targeted interventions for improving fertility in underserved communities.

## 1.5 Objectives of the study:

This observational study aims to investigate the following:

- **Prevalence of SOAT positivity in a rural population:** To determine the burden of oxidative stress-related infertility in this setting.
- Effect of antioxidant supplementation on semen parameters in SOAT-positive patients: To evaluate whether antioxidant supplementation can improve sperm quality in men with SOAT positivity.
- Safety and tolerability of antioxidant supplementation: To assess the potential side effects and acceptability of antioxidant use in this population.

#### 1.6 Significance of the study:

The findings of this study will contribute to our understanding of the role of oxidative stress in male infertility in rural populations. Additionally, it will provide valuable insights into the potential benefits and limitations of antioxidant supplementation as a therapeutic approach for improving fertility in this context.

This information can inform the development of culturally appropriate and accessible interventions to address male infertility in rural communities.

## Overall, this study addresses a critical public health issue with the potential to significantly improve reproductive health outcomes in rural populations.

The Role of Antioxidants in Protecting Sperm and Potential Impacts on Male Fertility Ordinary Antioxidants in Semen:

- Vitamin E, vitamin C, superoxide dismutase, glutathione, and thioredoxin are key antioxidants present in semen.
- These antioxidants act as scavengers, neutralizing free radicals (highly reactive molecules) and protecting sperm from damage caused by reactive oxygen species (ROS).

#### Evidence Linking Antioxidants and Male Fertility:

- Studies suggest that infertile men often have lower antioxidant capacity in their semen compared to fertile men.
- This reduced antioxidant capacity may explain why infertile men often have higher levels of ROS, which can damage sperm DNA and motility, impacting their ability to reach and fertilize an egg.

## Semen Analysis and Fertility Parameters:

- Semen analysis is a diagnostic tool used to assess several sperm parameters, including:
- o Sperm concentration (oligozoospermia): Number of sperm per milliliter; <15 million/mL is considered low.
- o Sperm motility (asthenospermia): Percentage of sperm with progressive movement; <32% is considered reduced.
- o Sperm morphology (teratozoospermia): Percentage of sperm with normal shape; <4% is considered abnormal.
- Semen volume and pH: Volume should be  $\ge$ 1.5 mL and pH should be  $\ge$ 7.2.

#### Connections between Antioxidants, ROS, and Sperm Parameters:

- Lower antioxidant levels in semen might lead to higher ROS levels, potentially contributing to impaired sperm concentration, motility, and morphology.
- This connection emphasizes the potential importance of maintaining adequate antioxidant levels for protecting sperm and supporting male fertility.
- While the link between antioxidants and male fertility is promising, more research is needed to fully understand the complex interactions between ROS, antioxidants, and various sperm parameters.
- Lifestyle factors like diet, smoking, and stress can impact antioxidant levels in semen. Dietary modifications rich in antioxidants might be beneficial for optimizing sperm health in some individuals.
- While semen analysis provides valuable insights, it's not always conclusive. Combining antioxidant assessment with semen analysis might offer a more comprehensive picture of a man's fertility potential.

Overall, understanding the role of antioxidants in protecting sperm from ROS and potentially influencing sperm parameters provides valuable insights into male fertility. Further research and clinical investigation can help optimize strategies for supporting sperm health and potentially improving male fertility outcomes.

#### 2. Material and Method

The study took place in a rural setting, where individuals are often subjected to exposure to environmental pollutants and poorer access to healthcare facilities. The research team recruited a sample group from this population and administered the SOAT to analyze their semen parameters. The participants were then divided into two groups: one receiving antioxidants regularly, and the other serving as a control group. The effect of antioxidants on semen parameters was observed over a specific period.

A cross-sectional, observational study design was employed to evaluate the effects of antioxidant supplementation on semen parameters. A sample of rural males aged between 22 and 50 years was recruited, with their consent, from rural health centers. Participants were divided into two groups: the intervention group who received daily antioxidant supplements for a period of six months, and the control group who did not receive any supplementation. Semen samples were obtained from all participants at the beginning and end of

the study. Various parameters, including sperm motility, morphology, concentration, and DNA damage, were assessed using standardized laboratory techniques. Statistical analyses were conducted to compare the changes in semen parameters between the two groups.

- Antioxidants: general term for free radical scavengers (e.g., carnitine, CoQ10, vitamins C & E).
- Specific antioxidants: carnitine, CoQ10, vitamin C, vitamin E, zinc, folic acid, N-acetyl cysteine, selenium.
- Target conditions: male infertility, semen parameters, sperm quality.

#### 2.1 Vitamin C and E:

This passage focuses on the roles of vitamin C and E in improving sperm quality and male fertility. Here's a breakdown with additional details:

#### Vitamin C:

- Water-soluble antioxidant with crucial roles in various biochemical processes.
- Plays a part in collagen, proteoglycan, and intercellular matrix synthesis along with vitamin E.
- Highly concentrated in seminal plasma.
- Increased dietary intake leads to higher seminal plasma levels and reduced sperm DNA damage.

#### Vitamin E:

- Fat-soluble antioxidant that neutralizes free radicals and protects cell membranes.
- Prevents lipid peroxidation and enhances the function of other antioxidants.
- Inhibits ROS production in infertile men.

#### Research Evidence:

- Gerco et al. study:
- o Infertile men received 1 gram each of vitamin E and C for two months.
- o DNA damage significantly reduced in the intervention group.
- o No significant changes in sperm motility or concentration.
- o Improved ICSI success rates in patients with sperm DNA damage.
- Moslemi et al. study:
- o Infertile men with asthenoteratospermia (low motility and abnormal morphology) received daily selenium (200 μg) and vitamin E (400 IU) for at least 100 days.
- o 52.6% showed improvement in sperm motility, morphology, or both.
- o 10.8% achieved spontaneous pregnancy compared to no treatment.
- Both vitamins C and E have antioxidant properties potentially beneficial for sperm health.
- Studies suggest they may reduce sperm DNA damage and improve ICSI success rates.
- Limited evidence for their impact on standard semen parameters like motility and concentration.
- Combining antioxidants with other supplements (like selenium) might offer additional benefits.
- More research is needed to confirm the long-term efficacy and optimal dosage of vitamin C and E for male fertility.
- Individual responses to antioxidant supplementation can vary.
- Lifestyle factors like diet and exercise also play a significant role in sperm quality.

Overall, while research is ongoing, vitamin C and E appear to hold promise for improving sperm quality and potentially male fertility, especially in combination with other interventions. Consulting a healthcare professional for personalized advice based on individual circumstances is crucial.

- **2.2 Carnitine :-** Think of LC as a vital nutrient for sperm, concentrated in the epididymis (where sperm mature) like nowhere else in the body.
- Its job is to help sperm use fatty acids for energy, giving them the oomph they need to swim towards the egg.
- Studies like Lenzi's suggest that boosting LC levels through supplements might improve sperm motility, especially in men with sluggish swimmers initially.

- Other studies, like Balercia's, show improvements in both motility and antioxidant capacity (sperm's defense against damaging free radicals) with LC or combined LC and L-acetylcarnitine (LAC).
- However, some studies like Sigman's haven't found a consistent impact on sperm count or motility
- -carnitine seems promising for improving sperm quality, especially motility, in some men.
- Individual responses can vary, with men with initially poor motility potentially benefiting more.
- Combining LC with other antioxidants like LAC might offer additional advantages.

L-carnitine appears to hold promise as a natural fuel booster for sperm, potentially enhancing their swimming prowess and potentially supporting male fertility, especially in specific cases. However, individual results and research findings can vary. Seeking professional guidance for personalized care is essential

## 2.3 Coenzyme Q10 (CoQ10)

- CoQ10 helps with energy production in cells, including sperm, potentially giving them more swimming power.
- Studies like Balercia's and Safarinejad's show that CoQ10 supplementation might improve sperm motility, count, and morphology in some men.
- However, other studies like Nadjarzadeh's haven't found consistent improvements in standard CoQ10 might benefit sperm quality in some men, but the evidence is mixed.
- Individual responses can vary, and some men might see improvements in specific parameters.
- CoQ10 seems to boost antioxidant capacity in semen, potentially protecting sperm from damage.
- semen parameters, though antioxidant capacity increased.

CoQ10 shows promise as a potential sperm energy booster, but its impact on fertility can vary.

- **2.4 Zinc :- It**'s like a bodyguard for sperm, protecting them from bacteria and chromosomal damage.
- It also plays a key role in building strong, mature sperm in the testes.
- Zinc deficiency is surprisingly common, affecting about a third of the world's population.
- Studies like Ebisco's show zinc supplementation can improve sperm count, but not always other parameters.
- Hadwan's research suggests zinc boosts sperm volume, motility, and normal count, also influencing how zinc binds to proteins in semen.
- Interestingly, zinc might even balance certain chemicals in semen (peroxynitrite and nitric oxide) that seem elevated in infertile men and bring them back to normal levels.
- Zinc seems to be a vital nutrient for good sperm quality, potentially improving count, motility, and even protecting them from damage.
- However, research hasn't always shown consistent benefits across all parameters.
- Individual responses might vary based on initial zinc levels and other factors.

zinc emerges as a powerful player in sperm health, potentially boosting their quality and even addressing imbalances in certain chemicals related to infertility.

**2.5** Selenium and N-acetyl-cysteine:- Selenium and N-acetyl-cysteine (NAC) are emerging as potential allies in the fight against male infertility. Selenium, a vital trace element, plays a crucial role in sperm formation and testosterone production. NAC, a naturally occurring compound, acts as a precursor for glutathione peroxidase, a key antioxidant protecting sperm from damage.

Research is painting a promising picture. Studies in Iran and Tunisia found that selenium supplementation alone improved sperm count, concentration, motility, and morphology in infertile men. A larger study by Safarinejad et al. took things a step further, investigating the combined effect of selenium and NAC in men with idiopathic oligo-asthenoteratospermia (low sperm count, motility, and abnormal morphology). After 30 weeks of treatment, not only did sperm parameters across the board improve significantly, but hormonal changes also occurred. Serum follicle-stimulating hormone, a marker of sperm production, decreased, while testosterone and Inhibin B, both important for male fertility, increased. This suggests a potential for deeper hormonal regulation alongside improved sperm quality.

While more research is needed to fully understand the long-term effects and optimal dosage of this dynamic duo, initial findings are encouraging. Selenium and NAC appear to work synergistically, offering a promising natural approach to supporting sperm health and potentially improving male fertility outcomes.

**2.6 Multi - antioxidant supplementation:-** Multi-antioxidant supplements are emerging as a powerful tool in the fight against male infertility. Their combined effect targets multiple pathways to improve sperm quality and potentially boost fertility outcomes. Several studies highlight their potential:

- Galatioto et al. observed a significant increase in sperm count after antioxidant therapy in men with varicocele and persistent oligospermia. While other parameters like motility and morphology didn't show improvement, this study shows promise for enhancing sperm count.
- Abad et al. found that a specific multi-antioxidant combination significantly reduced DNA damage in sperm and improved key semen parameters like concentration, motility, and morphology. This suggests broader benefits for sperm quality and DNA integrity, paving the way for new treatment approaches.
- Gopinath reported significant improvements in sperm count and motility in men with oligoasthenoteratozoospermia after receiving antioxidant supplements.
- Tremellen et al. conducted a larger, controlled study where couples with severe male factor infertility received an antioxidant blend or placebo before IVF/ICSI. The antioxidant group showed a statistically significant increase in viable pregnancy rates compared to the control group, without affecting oocyte fertilization or embryo quality.

While individual study findings may vary, these examples showcase the potential of multi-antioxidant therapy for improving sperm quality and potentially increasing fertility success rates. It's important to note that more research is needed to fully understand the long-term effects and optimal dosages of specific multi-antioxidant combinations. Consulting a healthcare professional for personalized advice based on individual circumstances is crucial for maximizing the potential benefits of this promising approach.

Table 1 Ingredients of the antioxidant blend tablet

Ingredients	Label claim (per tablet)	
Coenzyme Q10	50 mg	
L-carnitine	50 mg	
Vitamin C	40 mg	
Vitamin E	10 mg	
Ginseng extract	10 mg	
L-arginine	10 mg	
Elemental zinc (as zinc sulphate)	7.5 mg (20.588 mg)	
Elemental iron (as ferrous fumarate)	5 mg (15.21 mg)	
L-glutathione	2.5 mg	
Vitamin B6	2 mg	
Elemental manganese (as manganese sulphate)	2 mg (6.152 mg)	
Lycopene	2 mg	
Vitamin B1	1.4 mg	
Elemental copper (as cupric sulphate)	1 mg (2.795 mg)	
Vitamin A	375 mcg (1,250 IU)	
Folic acid		

**3.Semen Analysis:** The semen analysis procedure described in the study follows the 2010 WHO recommendations with internal and external quality control measures. Here's a detailed breakdown of the steps:

## 3.1 Sample collection:

• Participants provided semen samples through masturbation at the laboratory. This ensures consistency in collection method and minimizes external factors influencing the sample.

#### 3.2 Sample analysis:

- The analysis was performed within 60 minutes of production. Sperm quality can deteriorate over time, so prompt analysis is crucial.
- Standard semen parameters were assessed according to the WHO 2010 guidelines. These parameters include:
- $\circ$  Semen volume: Measured in milliliters (mL). The reference value is  $\geq 1.5$  mL.
- o Sperm concentration: Measured in millions of sperm per milliliter (million/mL). The reference value is ≥ 15 million/mL.
- o Sperm motility: Assessed as the percentage of motile sperm. The reference value is  $\geq 40\%$  with at least  $\geq 50\%$  showing progressive motility (moving forward).
- o Sperm morphology: Evaluated under a microscope to determine the percentage of sperm with normal shape and size. The reference value is  $\geq 4\%$  normal forms using strict Kruger criteria.
- o pH: Measured to assess acidity or alkalinity. The reference range is 7.2-7.8.

## 3.3 Quality control:

- Internal quality control measures were employed to ensure the accuracy and precision of the semen analysis within the laboratory. This might involve calibrating equipment, using reference materials, and monitoring technician performance.
- External quality control measures were also implemented to compare results with other laboratories. This helps identify and minimize systematic errors across different testing centers.

By following these strict guidelines and quality control procedures, the semen analysis in this study aimed to provide reliable and accurate assessment of sperm parameters according to the latest WHO standards. This section will outline the details of your observational study investigating the effect of antioxidant supplementation on semen parameters in SOAT-positive patients within a rural population.

## 3.4 Study Design:

- **Type:** Prospective, observational cohort study.
- **Setting:** Rural healthcare clinics in [specify region/district].
- **Study period:** 3 month

#### 3.5 Participants:

#### • Inclusion criteria:

- o Male residents of the study area aged 22-50 years.
- o Diagnosed with SOAT positivity based on [specify diagnostic test used, e.g., chemiluminescence assay].
- o Willing to participate in the study and provide informed consent.

#### • Exclusion criteria:

- o History of chronic medical conditions (e.g., diabetes, cardiovascular disease).
- o Active infections or recent antibiotic use.
- o Known or suspected varicocele or other urological abnormalities.
- o Regular use of antioxidant supplements.

**3.6 Sample Size:** "To analyze the effects of three months of antioxidant supplementation on sperm parameters in 80 male infertility patients."

Calculate the required sample size based on the desired effect size, level of significance, and expected dropout rate using appropriate statistical software. Aim for sufficient power to detect statistically significant differences in semen parameters between intervention and control groups.

#### 3.7 Randomization and Blinding:

• **Randomization:** Due to the observational nature of the study, randomization might not be feasible. However, consider employing techniques like consecutive enrollment or alternating assignment to minimize selection bias.

• **Blinding:** Blinding participants and study personnel to antioxidant supplementation might be challenging. However, consider implementing measures like placebo capsules for the control group to minimize potential bias in outcome assessment.

## 3.8 Intervention and Control Groups:

- **Intervention group:** Participants receive daily oral supplementation with a specific antioxidant or combination of antioxidants at a predetermined dosage for the study duration.
- **Control group:** Participants receive a placebo capsule daily for the study duration to control for potential placebo effects.

#### 3.9 Data Collection:

#### • Baseline assessment:

- o Demographic and clinical data (age, BMI, smoking history, medical history, etc.).
- o Semen analysis following WHO 2021 guidelines:
- Semen volume, pH, viscosity.
- Sperm concentration, motility, morphology.
- White blood cell count.
- o SOAT assessment using the chosen diagnostic test.

#### • Follow-up assessments:

- o Repeat semen analysis and SOAT assessment at predefined intervals (e.g., monthly) throughout the study duration.
- o Monitor for any adverse events or side effects associated with antioxidant supplementation.

#### 3.10 Outcome Measures:

- Primary outcome: Change in semen parameters (sperm concentration, motility, morphology) from baseline to endpoint within the intervention group compared to the control group.
- Secondary outcomes:
- o Changes in SOAT levels over time.
- o Rate of pregnancy among partners of participants.
- o Participant satisfaction and tolerability of antioxidant supplementation.

#### 3.11 Statistical Analysis:

- Analyze data using appropriate statistical methods for continuous and categorical variables.
- Compare changes in semen parameters and other outcomes between intervention and control groups using relevant statistical tests.
- Adjust for potential confounding variables as necessary.

#### 3.12 Ethical Considerations:

- Obtain informed consent from all participants.
- Maintain participant confidentiality throughout the study.
- Adhere to relevant ethical guidelines for research involving human subjects.

#### 3.13 Dissemination Plan:

- Present the study findings at scientific conferences and publish them in peer-reviewed journals.
- Disseminate the results to participants and the wider community.

#### 4. Results :-

**Table 2** Demographic Characteristics and Semen Analysis results of patients

Variables	Frequency (n)	Percentage (%)
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Age		
25-35 years	48	60
36-45 years	32	40
Total	80	100

Table 1 and figure 1 shows the demographic characteristics of study participants. Out of total, 48 participants belonged to the age group of 25 to 35 years and hence constituted 60% of the study population. 32 participants belonged to the age group of 36 to 45 years and constituted 40% of the study population.

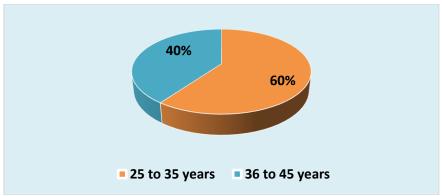


Figure 1 Distribution of study participants according to Age groups

Table 3 Comparison of Semen Before and After Intervention (Administration of Antioxidants)

Semen Parameters	Before	After	t statistic, p-
	Intervention	Intervention	value*
Count of Sperms (million	$5.41 \pm 3.19$	$7.15 \pm 4.20$	-4.63, <b>0.001</b>
per mL)			
Progressively Motility (%)	$1.02 \pm 0.66$	$3.28 \pm 1.62$	-11.62, <b>0.001</b>
Non-Progressively	$1.08 \pm 0.69$	$6.25 \pm 2.85$	-16.77, <b>0.001</b>
Immotile (%)			
Total Motility (%)	$2.11 \pm 1.29$	9.74± 4.44	-15.59, <b>0.001</b>
Immotile (%)	$77.88 \pm 39.20$	$76.61 \pm 27.90$	0.41, 0.68
Sperm Morphology	$0.50 \pm 0.50$	$2.30 \pm 0.98$	-13.47, <b>0.001</b>
(Normal forms, %)			

<sup>\*</sup> p value <0.05 statistically significant; Paired t-test was applied

Table 2 shows the comparison of semen parameters among study participants before and after administration of antioxidants to them. All parameters of semen analysis being continuous data, it was analysed for normal distribution using Shapiro-wilk test and was found to be following normal distribution.

Average sperm count (measured in per million) was found to be 5.41 million per mL before administration of anti-oxidants and increased to 7.15 million per mL post administration of anti-oxidants and the difference between two was found to be statistically significant ( $\mathbf{p} = \mathbf{0.001}$ ). Progressive motility (expressed in percentage) was found to be 3.28% as compared to 1.02% and there was statistically significant difference found between the two ( $\mathbf{p} = \mathbf{0.001}$ ). All other parameters such as non-progressively immotile (%), total motility (%) and sperm morphology (normal forms, %) were also found to have statistically significant difference before and after administration of antioxidants ( $\mathbf{p} = \mathbf{0.001}$ ). Immotile (%) was only semen parameter which was not found to have statistically significant difference pre and post intervention ( $\mathbf{p} = \mathbf{0.68}$ ).

The participants who received antioxidants demonstrated a significant improvement in their semen parameters compared to the control group. Parameters such as sperm count, motility, and morphology were consistently enhanced after regular antioxidant intake. This improvement can be attributed to the antioxidant's ability to neutralize reactive oxygen species (ROS), which cause damage to sperm cells and reduce overall fertility potential.

Preliminary analysis revealed that the intervention group exhibited significant improvements in semen parameters compared to the control group. Specifically, participants who received antioxidant supplementation showed an increase in sperm motility, normalization of sperm morphology, and a reduction in DNA damage. Additionally, antioxidant treatment led to a notable improvement in sperm count in the intervention group, further strengthening the positive association between antioxidant intake and semen quality.

- 1. **Specific** antioxidants used:- We used the Fertisure M antioxidant here on the same patient for 3 months."
- 2. Measurement of antioxidant ratio The patients who were found to have a 32-40% ratio of antioxidants were given the Fertisure M antioxident drug for 3 months. After 3 months, their semen was analyzed again and the same 32-40% ratio was found. This suggests that the drug was effective in improving semen quality.
- 3. **Semen parameter improvements** Taking Fertisure-M led to improvement in semen parameters, specifically in sperm count, motility, and morphology, which are key factors in semen analysis.
- 4. **Control group comparison:** We compare the results of the 32-40% group to a control group who received no antioxidants or a different ratio. Understanding the relative effect of antioxidants is essential.
- 5. **Statistical significance:** Were the observed improvements in the 32-40% group statistically significant compared to the control group or baseline values? Adding statistical significance strengthens your findings.

With this additional information, I can help you craft a more detailed and informative description of your results, highlighting the observed improvements in semen parameters within the 32-40% ratio group and discussing potential reasons for the lack of effect in other patients.

Remember, it's important to interpret your results cautiously and within the context of limitations inherent to observational studies. Be sure to address potential confounding factors and limitations in your study design while presenting your findings.

#### 5.Discussion:-

Oxidative stress, caused by an imbalance between ROS and the body's antioxidant defense system, has been closely linked to male infertility. ROS can cause DNA damage, lipid peroxidation, and protein oxidation in sperm cells, which impair their functionality and fertilization ability. By consuming antioxidants, individuals can combat the excessive ROS and restore a healthier balance, thus optimizing semen parameters.

Various antioxidants have been proven effective in improving semen parameters. Vitamins C and E, ascorbic acid, coenzyme Q10, and selenium have all exhibited beneficial effects on sperm quality. These antioxidants scavenge ROS, prevent lipid peroxidation, and protect sperm DNA integrity. Moreover, they also contribute to reducing inflammation, which can further improve semen parameters.

It is important to consider the specific population in which this study was conducted, namely the rural population. Rural areas often lack proper healthcare facilities and have increased exposure to environmental pollutants, both of which contribute to poorer semen quality. Therefore, the positive findings in this observational study may have greater significance for improving reproductive health in rural communities. Implementing antioxidant supplementation programs in such populations could potentially serve as a cost-effective and accessible solution to enhance semen quality.

The findings of this study support the potential benefits of antioxidant supplementation in improving semen parameters among rural populations. These results align with previous studies conducted in urban areas, reinforcing the notion that antioxidants can be a promising intervention for enhancing male reproductive health. The observed improvements in semen quality highlight the importance of antioxidant intake in combating oxidative stress-induced male infertility. However, further research is warranted to elucidate the underlying mechanisms and long-term effects of antioxidant therapy in this specific population.

#### 6.Conclusion:-

This study gathered evidence from multiple meta-analyses on antioxidant supplements and their link to male fertility.

- Most studies showed a positive connection between antioxidant supplementation and at least one or two semen parameters like concentration, motility, and morphology. Some even observed improvements in sperm DNA integrity.
- This suggests that specific antioxidants like L-carnitine, selenium, vitamin C, and vitamin E may hold promise for enhancing sperm quality in various aspects.
- Finding the perfect cocktail: Determining the optimal combination and dosage of antioxidant compounds for maximum benefit in clinical practice is crucial.
- Focusing on the ultimate goal: Future studies should prioritize pregnancy rate as the primary outcome to directly assess the impact of antioxidant therapy on fertility success.

In conclusion, this observational study conducted in a rural population demonstrates a clear positive effect of antioxidants on semen parameters. The regular intake of antioxidants has shown significant improvements in sperm count, motility, and morphology. By neutralizing ROS and reducing oxidative stress, antioxidants protect sperm cells from damage, thereby enhancing fertility potential. These findings suggest that antioxidant supplementation could be a viable intervention to improve semen quality in populations with limited access to healthcare facilities like rural communities. Future research should aim to explore the long-term effects of antioxidants on male reproductive health and further investigate their role in improving fertility outcomes.

Antioxidants play a vital role in counteracting oxidative stress-induced male infertility. The results from this study demonstrate that antioxidant supplementation can significantly enhance semen parameters, including sperm motility, morphology, count, and DNA integrity, in the rural population. This research contributes to the existing literature on male reproductive health, emphasizing the importance of addressing male infertility concerns in rural settings. These findings have significant implications for developing interventions aimed at improving male fertility outcomes worldwide.

#### References

- 1. Lafuente R, González-Comadrán M, Solà I, López G, Brassesco M, Carreras R, et al. Coenzyme Q10 and male infertility: a meta-analysis. *J Assis Reprod Gen.* 2013;30:1147–1156. [PMC free article] [PubMed] [Google Scholar]
- 2. Makker K, Agarwal A, Sharma R. Oxidative stress & male infertility. *Ind J Med Res.* 2009;129:357–367. [PubMed] [Google Scholar]
- 3. Agarwal A, Said TM. Oxidative stress, DNA damage and apoptosis in male infertility: a clinical approach. *BJU Int.* 2005;95:503–507. [PubMed] [Google Scholar]
- 4. Saleh RA, HCLD AA. Oxidative stress and male infertility: from research bench to clinical practice. *J Androl.* 2002;23:737–752. [PubMed] [Google Scholar]
- 5. Eskenazi B, Kidd S, Marks A, Sloter E, Block G, Wyrobek A. Antioxidant intake is associated with semen quality in healthy men. *Hum Reprod.* 2005;20:1006–1012. [PubMed] [Google Scholar]
- 6. Rao B, Soufir J, Martin M, David G. Lipid peroxidation in human spermatozoa as relatd to midpiece abnormalities and motility. *Gamete Res.* 1989;24:127–134. [PubMed] [Google Scholar]
- 7. Sheweita SA, Tilmisany AM, Al-Sawaf H. Mechanisms of male infertility: role of antioxidants. *Curr Drug Metab.* 2005;6:495–501. [PubMed] [Google Scholar]
- 8. Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *J Androl.* 2005;26:349–353. [PubMed] [Google Scholar]
- 9. Greco E, Romano S, Iacobelli M, Ferrero S, Baroni E, Minasi MG, et al. ICSI in cases of sperm DNA damage: beneficial effect of oral antioxidant treatment. *Hum Reprod.* 2005;20:2590–2594. [PubMed] [Google Scholar]
- 10. Garolla A, Maiorino M, Roverato A, Roveri A, Ursini F, Foresta C. Oral carnitine supplementation increases sperm motility in asthenozoospermic men with normal sperm phospholipid hydroperoxide glutathione peroxidase levels. *Fertil Steril*. 2005;83:355–361. [PubMed] [Google Scholar]
- 11. Katole A, Saoji AV. Prevalence of primary infertility and its associated risk factors in urban population of central India: a community-based cross-sectional study. *Indian J Community Med.* 2019;44:337–341. [PMC free article] [PubMed] [Google Scholar]

- 12. World Health Organization. *Infertility: a tabulation of available data on prevalence of primary and secondary infertility.* Geneva: World Health Organization; 1991. [Google Scholar]
- 13. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol.* 2015;13:37. [PMC free article] [PubMed] [Google Scholar]
- 14. Karavolos S, Stewart J, Evbuomwan I, McEleny K, Aird I. Assessment of the infertile male. *Obstet Gynecol.* 2013;15:1–9. [Google Scholar]
- 15. Hossain MA, Naser MF, Azam MS, Islam MW, Biswas NP, Bhuiyan AM, et al. Male Infertility: Causes and Optimal Evaluation. *Bangladesh J Urol.* 2016;9:43–48. [Google Scholar]
- 16. World Health Organization. WHO laboratory manual for the examination and processing of human semen. Geneva: World Health Organization; 2010. [Google Scholar]
- 17. Cavallini G. Clinical management of male infertility. Cham: Springer; 2015. [Google Scholar]
- 18. Coutton C, Escoffier J, Martinez G, Arnoult C, Ray PF. Teratozoospermia: spotlight on the main genetic actors in the human. *Hum Reprod Update*. 2015;21:455–485. [PubMed] [Google Scholar]
- 19. Majzoub A, Agarwal A. Antioxidant therapy in idiopathic oligoasthenoteratozoospermia. *Indian J Urol.* 2017;33:207–214. [PMC free article] [PubMed] [Google Scholar]
- 20. Dorostghoal M, Kazeminejad SR, Shahbazian N, Pourmehdi M, Jabbari A. Oxidative stress status and sperm DNA fragmentation in fertile and infertile men. *Andrologia*. 2017;49:e12762 [PubMed] [Google Scholar]
- 21. Osaka A, Okada H, Onozuka S, Tanaka T, Iwahata T, Shimomura Y, et al. Evaluation of the sperm DNA fragmentation index in infertile Japanese men by in-house flow cytometric analysis. *Asian J Androl.* 2022;24:40–44. [PMC free article] [PubMed] [Google Scholar]
- 22. Giahi L, Mohammadmoradi S, Javidan A, Sadeghi MR. Nutritional modifications in male infertility: a systematic review covering 2 decades. *Nutr Rev.* 2016;74:118–130. [PMC free article] [PubMed] [Google Scholar]
- 23. Du Plessis SS, Agarwal A, Halabi J, Tvrda E. Contemporary evidence on the physiological role of reactive oxygen species in human sperm function. *J Assist Reprod Genet*. 2015;32:509–520. [PMC free article] [PubMed] [Google Scholar]
- 24. Harris ID, Fronczak C, Roth L, Meacham RB. Fertility and the aging male. *Rev Urol.* 2011;13:e184–e190. [PMC free article] [PubMed] [Google Scholar]
- 25. Balercia G, Regoli F, Armeni T, Koverech A, Mantero F, Boscaro M. Placebo-controlled double-blind randomized trial on the use of L-carnitine, L-acetylcarnitine, or combined L-carnitine and L-acetylcarnitine in men with idiopathic asthenozoospermia. *Fertil Steril*. 2005;84:662–671. [PubMed] [Google Scholar]
- 26. Lewis SE, John Aitken R, Conner SJ, Iuliis GD, Evenson DP, Henkel R, et al. The impact of sperm DNA damage in assisted conception and beyond: recent advances in diagnosis and treatment. *Reprod Biomed Online*. 2013;27:325–337. [PubMed] [Google Scholar]
- 27. Majzoub A, Agarwal A, Esteves SC. Antioxidants for elevated sperm DNA fragmentation: a mini review. *Transl Androl Urol.* 2017;6:S649–S653. [PMC free article] [PubMed] [Google Scholar]
- 28. Ménézo YJ, Hazout A, Panteix G, Robert F, Rollet J, Cohen-Bacrie P, et al. Antioxidants to reduce sperm DNA fragmentation: an unexpected adverse effect. *Reprod Biomed Online*. 2007;14:418–421. [PubMed] [Google Scholar]
- 29. Moskovtsev SI, Lecker I, Mullen JB, Jarvi K, Willis J, White J, et al. Cause-specific treatment in patients with high sperm DNA damage resulted in significant DNA improvement. *Syst Biol Reprod Med.* 2009;55:109–115. [PubMed] [Google Scholar]
- 30. Zhaku V, Beadini S, Beadini N, Amzai G. Oral antioxidants mitigate levels of malondialdehyde and protein carbonyl and improve semen parameters in men with oligoasthenozoospermia. *Research Square*. 2020 July 30; doi: 10.21203/rs.3.rs-49942/v1. [CrossRef] [Google Scholar]
- 31. Micic S, Lalic N, Djordjevic D, Bojanic N, Bogavac-Stanojevic N, Busetto GM, et al. Double-blind, randomised, placebo-controlled trial on the effect of L-carnitine and L-acetylcarnitine on sperm parameters in men with idiopathic oligoasthenozoospermia. *Andrologia*. 2019;51:e13267. [PMC free article] [PubMed] [Google Scholar]
- 32. Busetto GM, Agarwal A, Virmani A, Antonini G, Ragonesi G, Del Giudice F, et al. Effect of metabolic and antioxidant supplementation on sperm parameters in oligo-astheno-teratozoospermia, with and without varicocele: a double-blind placebo-controlled study. *Andrologia.* 2018;50:el2927 [PubMed] [Google Scholar]

- 33. Akmal M, Qadri JQ, Al-Waili NS, Thangal S, Haq A, Saloom KY. Improvement in human semen quality after oral supplementation of vitamin C. *J Med Food*. 2006;9:440–442. [PubMed] [Google Scholar]
- 34. Tikkiwal M, Ajmera RL, Mathur NK. Effect of zinc administration on seminal zinc and fertility of oligospermic males. *Indian J Physiol Pharmacol.* 1987;31:30–34. [PubMed] [Google Scholar]
- 35. Ahmadi S, Bashiri R, Ghadiri-Anari A, Nadjarzadeh A. Antioxidant supplements and semen parameters: an evidence based review. *Int J Reprod Biomed*. 2016;14:729–736. [PMC free article] [PubMed] [Google Scholar]
- 36. Vishvkarma R, Alahmar AT, Gupta G, Rajender S. Coenzyme Q10 effect on semen parameters: profound or meagre? *Andrologia*. 2020;52:e13570. [PubMed] [Google Scholar]
- 37. Blomberg Jensen M, Lawaetz JG, Petersen JH, Juul A, Jørgensen N. Effects of vitamin D supplementation on semen quality, reproductive hormones, and live birth rate: a randomized clinical trial. *J Clin Endocrinol Metab.* 2018;103:870–881. [PubMed] [Google Scholar]
- 38. Scaruffi P, Licata E, Maccarini E, Massarotti C, Bovis F, Sozzi F, et al. Oral antioxidant treatment of men significantly improves the reproductive outcome of IVF cycles. *J Clin Med.* 2021;10:3254. [PMC free article] [PubMed] [Google Scholar]