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The Role of Rhodanese Enzyme in Oxidative Stress Modulation and its Impact on Sperm Quality and Male Fertility

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ABSTRACT

Oxidative stress is a key factor affecting male reproductive health, leading to impaired sperm quality and increased DNA damage. Antioxidant systems play a crucial role in maintaining sperm integrity and functionality. This study investigates the relationship between a specific enzyme activity, oxidative stress markers, and sperm quality parameters to understand its potential protective role in reproductive health.

Seminal plasma samples were analyzed for enzymatic activity, antioxidant capacity, lipid peroxidation levels, and DNA fragmentation. Correlation analyses and statistical tests were conducted to evaluate the associations between these factors.

A strong positive correlation was observed between enzymatic activity and antioxidant capacity, along with significant negative correlations with lipid peroxidation and DNA fragmentation. These findings highlight the enzyme's role in mitigating oxidative stress-related damage to sperm.

The enzyme studied appears to play a protective role in reducing oxidative damage and improving sperm quality. Enhancing its activity could offer a potential therapeutic approach to addressing male reproductive health challenges.

Keywords: Oxidative stress, sperm quality, antioxidant systems, DNA damage, male reproductive health, lipid peroxidation, fertility enhancement.

1. Introduction

1.1 Overview of Male Infertility

- Definition, prevalence, and major causes.

Male infertility is defined as the inability of a couple to achieve pregnancy after 12 months of regular, unprotected intercourse [1]. Male factors contribute to approximately 40% of infertility cases globally, with a similar proportion attributable to female factors and the remaining 20% to combined or unexplained causes [2]. Major causes of male infertility include infections, trauma, environmental toxin exposure, anatomical anomalies, chromosomal abnormalities, systemic illnesses, and immune responses such as anti-sperm antibodies. Lifestyle factors, including smoking, alcohol use, obesity, and advanced age, also increase risk [3].

- Importance of identifying molecular mechanisms affecting sperm quality.

Despite advances in diagnostic tools, 40%–50% of male infertility cases remain idiopathic [4]. Understanding the molecular mechanisms, particularly those affecting sperm quality, is essential for developing targeted diagnostic and therapeutic approaches. Exploring factors such as oxidative stress, genetic integrity, and sperm cell biochemistry is vital to improving fertility outcomes.

1.2 Role of Oxidative Stress in Male Infertility

Oxidative stress (OS) occurs when the balance between reactive oxygen species (ROS) production and antioxidant defense mechanisms is disrupted. ROS, including hydrogen peroxide (H2O2), superoxide anion (O2•–), and hydroxyl radicals (•OH), are by-products of cellular metabolism. While ROS serve physiological roles at controlled levels, excess ROS leads to cellular damage and compromises sperm function.

Impact of OS on sperm quality:

- **Lipid peroxidation:** The high content of polyunsaturated fatty acids in sperm membranes makes them particularly vulnerable to lipid peroxidation. This process compromises membrane integrity, leading to impaired motility and reduced fertilization capacity.

- **DNA fragmentation:** ROS induce oxidative damage to sperm DNA, leading to fragmentation that affects embryonic development and pregnancy success.
- **Motility issues:** ROS impair sperm motility by disrupting mitochondrial function and depleting intracellular ATP levels. Excessive ROS levels are strongly correlated with poor sperm performance and reduced fertility

1.3 Focus on Rhodanese Enzyme

Rhodanese is a mitochondrial enzyme known for its sulfur transferase activity, primarily involved in cyanide detoxification, biosynthesis of iron-sulfur clusters, and cellular redox balance. It plays a role in sulfur metabolism and antioxidant defense by facilitating interactions with thioredoxin and glutathione systems. Rhodanese activity has been detected in various tissues, including the male reproductive system.

Hypothesis:
Rhodanese is hypothesized to serve as a critical regulator in mitigating oxidative stress in the male reproductive system. By enhancing antioxidant capacity and reducing lipid peroxidation, rhodanese could play a protective role in maintaining sperm integrity and function. This potential function underscores its importance as a target for understanding and treating oxidative stress-induced male infertility.

The Necessity of Research

Male infertility is a global health concern, with approximately 15% of couples experiencing challenges in conceiving, and male factors contributing to nearly 40% of these cases. Despite advances in medical science, a significant proportion of male infertility cases remain idiopathic, emphasizing the need for deeper insights into the underlying molecular mechanisms. Among these mechanisms, oxidative stress (OS) has emerged as a critical factor impacting sperm quality through processes such as lipid peroxidation, DNA fragmentation, and compromised motility.

2. Literature Review

2.1 Mechanisms of Oxidative Stress in the Male Reproductive System

Oxidative stress (OS) in the male reproductive system is caused by an imbalance between the production of reactive oxygen species (ROS) and the ability of antioxidants to neutralize these harmful molecules. ROS are highly reactive molecules containing oxygen, including free radicals such as superoxide anion ($O_2^{\bullet-}$) and hydroxyl radicals ($OH^{\bullet}$), as well as non-radical molecules like hydrogen peroxide (H_2O_2). These ROS can be produced endogenously, primarily through mitochondrial respiration during ATP production, which generates ROS as byproducts [5].Additionally, exogenous sources such as environmental toxins, pollutants, smoking, alcohol consumption, and industrial chemicals contribute to increased ROS levels in semen, exacerbating oxidative damage [6].

ROS production in sperm is a natural process but can lead to sperm dysfunction when levels are excessively high. The oxidative damage caused by ROS manifests through several pathways, primarily lipid peroxidation, which compromises sperm membrane integrity. Lipid peroxidation results in the breakdown of polyunsaturated fatty acids in sperm membranes, increasing membrane permeability, disrupting motility, and leading to structural DNA damage[7]. DNA fragmentation and mitochondrial dysfunction also result from ROS-induced damage, further impairing sperm quality and contributing to male infertility[8]. The balance between ROS production and antioxidant defense mechanisms is therefore crucial for maintaining sperm health and fertility.

2.2 Role of Antioxidants in Combating Oxidative Stress

To protect cells from the harmful effects of ROS, organisms employ both enzymatic and non-enzymatic antioxidant defense systems. Enzymatic antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px) are crucial in mitigating oxidative stress. SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, while catalase and GSH-Px reduce hydrogen peroxide to water or alcohols, respectively [9]. These enzymes are abundant in sperm and act as the first line of defense against oxidative damage. However, research into the specific contributions of each enzyme to sperm protection is still developing. While much is known about GSH-Px and SOD, the precise roles and synergistic interactions of these enzymes in spermatozoa are not fully elucidated [10].

Non-enzymatic antioxidants, including vitamins C and E, glutathione (GSH), and trace elements like zinc, selenium, and copper, also play pivotal roles in neutralizing ROS. These antioxidants directly scavenge ROS and help maintain cellular redox balance. For example, vitamin E, a lipid-soluble antioxidant, protects the sperm membrane from lipid peroxidation, while vitamin C helps regenerate other antioxidants like vitamin E [11].However, while the importance of non-enzymatic antioxidants is recognized, gaps remain in understanding their cumulative effects on male fertility, especially in the context of antioxidant supplementation therapies [12].

2.3 Rhodanese: A Multifunctional Enzyme

Rhodanese, or thiosulfate sulfurtransferase (TST), is an enzyme that catalyzes the transfer of sulfur from a donor molecule, typically thiosulfate, to a nucleophilic acceptor. Initially identified for its role in cyanide detoxification, rhodanese is now recognized for its broader biochemical functions,

including its involvement in cellular sulfur metabolism and its potential antioxidant properties [13]. Rhodanese has been implicated in various physiological processes, such as the formation of iron-sulfur proteins, which are crucial for mitochondrial function and cellular energy production [14].

In the context of oxidative stress, rhodanese may serve an antioxidant-like function, helping to mitigate oxidative damage in non-reproductive tissues. The enzyme participates in sulfur transfer reactions, which could influence redox signaling and cellular defense mechanisms. It interacts with other antioxidant systems, such as the glutathione (GSH) system, to maintain cellular homeostasis [15]. Rhodanese has also been shown to regulate hydrogen sulfide (H2S) production, which has anti-inflammatory and cytoprotective effects, further suggesting its role in cellular stress [16].

Although the majority of rhodanese studies have focused on its role in non-reproductive systems, emerging research indicates that it may contribute to sperm quality by participating in redox regulation within spermatozoa. Rhodanese's ability to counteract oxidative damage by modulating sulfur metabolism and interacting with the GSH system places it as a potentially crucial player in protecting sperm from oxidative stress. However, more research is needed to clarify the exact mechanisms by which rhodanese may influence sperm health and its potential therapeutic applications in treating male infertility.

Article Title	**Author(s)**	**Conclusion**
Mechanisms of Oxidative Stress in the Male Reproductive System	[5]	This study discusses the sources of ROS, both endogenous (mitochondria) and exogenous (environmental toxins), and their link to sperm damage, highlighting oxidative stress.
The Role of Oxidative Stress in Sperm Dysfunction	[8]	The research concludes that oxidative stress plays a central role in sperm dysfunction, affecting motility, viability, and DNA integrity, which contribute to male infertility.
Role of Antioxidants in Male Infertility	[10]	The article emphasizes the importance of antioxidants, especially enzymatic ones like SOD and catalase, in protecting sperm from ROS-induced damage and improving fertility.
Sources and Consequences of Oxidative Stress in Male Infertility	[6]	Henkel et al. found that oxidative stress in semen is a significant contributor to male infertility, stressing the need for antioxidants in clinical treatments.
Effects of Vitamin E and C on DNA Damage in Sperm	[11]	Vitamin E and C supplementation reduces oxidative damage in sperm DNA, improving sperm quality in men with infertility.
The Effects of Antioxidants in Male Infertility Treatment	[12]	This review shows that antioxidants, including selenium and zinc, effectively reduce ROS levels, improve sperm quality, and support fertility treatments in men.
Effects of Vitamin C, E, and Glutathione on Sperm DNA Oxidation	[17]	After antioxidant treatment, sperm DNA oxidation levels significantly decreased, and sperm concentration improved in infertile men.
The Role of Rhodanese in Sulfur Metabolism and Oxidative Stress	[15]	Rhodanese is essential in cellular detoxification processes, including ROS regulation, and plays a protective role in reducing oxidative stress.
Rhodanese in Mitochondrial Function and Oxidative Stress	[13]	Rhodanese helps modulate oxidative stress by transferring sulfur groups, which aids in detoxifying ROS, thereby protecting cellular structures, including sperm.
Oxidative Stress and Male Fertility: The Role of Rhodanese	[16]	The study suggests that rhodanese may protect sperm quality by managing oxidative stress and sulfur metabolism, which are crucial for maintaining male fertility.
Oxidative Stress and Mitochondrial Dysfunction in Sperm Quality	[18]	This paper concludes that oxidative stress in sperm mitochondria directly affects sperm motility and DNA integrity, contributing to male infertility.
Impact of Sperm DNA Fragmentation on Male Fertility	[19]	DNA fragmentation in sperm, often exacerbated by oxidative stress, plays a major role in male infertility, affecting fertilization and embryo development.

The Role of Rhodanese in Oxidative Stress Modulation in Sperm	[20]	Rhodanese's activity in oxidative stress modulation is linked to protecting sperm against ROS-induced damage, improving sperm viability and fertility outcomes.
Role of Rhodanese in Sulfur-Containing Molecules and Antioxidant Defense	[14]	Rhodanese plays a critical role in sulfur transfer, which is key to maintaining antioxidant defenses and protecting sperm from oxidative damage.

3. Materials and Methods

3.1 Study Population/Samples

- **Description of Animal Samples:** The study involved six healthy and sexually mature male Qezel rams aged 3-4 years with an average weight of 45-55 kg. Sperm samples were collected biweekly (twice per week), and a total of five sperm samples were collected from each ram using an artificial vagina (AV).
- **Parameters Measured:** The following sperm parameters were evaluated: sperm motility, concentration, viability, and morphology. Additionally, markers of oxidative stress, including lipid peroxidation (measured via malondialdehyde, MDA), total antioxidant capacity (TAC), and DNA fragmentation, were assessed.

3.2 Experimental Procedures

- **Sperm Collection:** Sperm samples were collected from the rams using an artificial vagina (AV), ensuring optimal conditions such as temperature (40-43°C), internal pressure, and lubricity. The AV was cleaned, sterilized, and dried before use to prevent any contamination. Samples were collected twice a week, with five samples obtained per ram.

Figure 3-1: Artificial Vagina for Ram Sperm Collection

- **Post-Collection Handling:**
  - Samples were immediately transported in an insulated flask at 35-37°C to prevent cold shock.
  - Avoiding exposure to direct sunlight, as this can cause hydrogen peroxide production, damaging sperm motility and viability.
  - Care was taken to prevent any contact with water, as it leads to sperm mortality.
- **Sperm Parameters Measured:**

1. **Sperm Volume:** Measured using specialized collection tubes with a precision of 0.1 mL. The average volume in rams ranged from 0.7 mL to 2 mL, with an overall mean of 1 mL.

2. **Sperm Motility:** After dilution with 2.9% sodium citrate (1:100 ratio), sperm motility was assessed under a microscope at 400x magnification.

3. **Sperm Concentration:** Measured using a hemocytometer after dilution with sodium citrate. A formula was used to calculate the sperm concentration in each milliliter.

Table 3-1: List of Equipment and Materials Used

Item	**Country of Manufacture**
Spectrophotometer	China
Vortex	UK
Digital Balance	Japan
Water Bath	Iran
Pipettes	Germany
Glycine	UK
Sodium Cyanide	Merk (Germany)
Sodium Thiosulfate	Merk (Germany)

Formaldehyde	Merk (Germany)
Ferric Nitrate	Merk (Germany)
Tris	Merk (Germany)
DTNB	Merk (Germany)
Folin-Ciocalteu Solution	Merk (Germany)
Sodium Citrate	Merk (Germany)
Sodium Molybdate	Merk (Germany)

4. **Viability of Sperm:** Measured using the eosin-nigrosin staining method, where live sperm do not absorb eosin and dead sperm do. The proportion of viable and non-viable sperm was calculated based on the staining.

Table 3-2: Composition of Eosin-Nigrosin Staining Solution (in 100 mL Distilled Water)

Material	**Amount (grams)**
Eosin dye	0.67
Nigrosin dye	0.10
Sodium Chloride	0.90

5. **Sperm Morphology:** Evaluated using light microscopy at 400x magnification, focusing on sperm without tails, abnormal tails, and sperm with abnormal heads. A minimum of 200 sperm per sample were assessed.

6. **Wavy Motility:** This measure evaluates the swimming pattern and was scored from 0 (no movement) to 5 (rapid wave motion).

Table 3-3: Grading of Sperm Wavy Motility

Description	**Score**
Very fast wave-like motion (≥90% sperm active)	5
Fast wave-like motion (70%-85% active)	4
Moderate wave-like motion (45%-65% active)	3
Some wave motion (20%-40% active)	2
Minimal activity (≤10% sperm active)	1
No movement	0

7. **Lipid Peroxidation:** Assessed by measuring MDA formation, a product of lipid peroxidation, through spectrophotometry.

- **Separation of Seminal Plasma:** Seminal plasma was separated by centrifugation at 4000 RPM for 10 minutes.

3.3 Statistical Analysis

- **Statistical Tools:**
    - **Pearson's Correlation Test** was used to evaluate the relationships between rhodanese activity, lipid peroxidation, total antioxidants, and DNA fragmentation.
    - **ANOVA** and **Tukey's test** were used to analyze differences among groups for various sperm parameters and oxidative stress markers.
    - **SPSS-16** was used for all data analyses. The correlation coefficient (r) was categorized as follows:
        - **0.1-0.3**: Weak correlation
        - **0.3-0.5**: Moderate correlation
        - **>0.5**: Strong correlation.

4. Results

4.1 Sperm Parameters and Rhodanese Activity

- Correlation between rhodanese activity and sperm motility, morphology, and viability:

The correlation between rhodanese activity and sperm motility, morphology, and viability was assessed. As observed in **Table 4-1**, the sperm motility, sperm concentration, sperm viability, and sperm abnormalities were measured. The results indicated a **moderate positive correlation** between rhodanese activity and sperm motility (r = 0.451, p = 0.012). Similarly, a **negative correlation** was found between rhodanese activity and sperm abnormalities (r = -0.407, p = 0.025), suggesting that higher rhodanese activity may be associated with improved sperm quality.

The average rhodanese activity in semen samples from all six groups of rams was **1.95 ± 0.54 U/L**, with a range from **1.84 ± 0.55 U/L** to **2.43 ± 0.84 U/L**. This data suggests a consistent rhodanese activity level that may influence sperm parameters.

Ram Number	Abnormal Sperm (%)	Semen Volume (mL)	Sperm Motility (%)	Sperm Concentration (×10⁹ per mL)	Sperm Viability (%)
1	4.22 ± 1.09	1.16 ± 0.13	66.10 ± 10.23	1.64 ± 0.17	78.64 ± 11.22
2	3.59 ± 0.98	1.03 ± 0.17	55.30 ± 8.77	1.58 ± 0.32	65.57 ± 6.76
3	3.15 ± 0.86	1.12 ± 0.15	54.42 ± 14.65	1.93 ± 0.10	69.04 ± 8.29
4	3.04 ± 0.95	1.32 ± 0.16	55.86 ± 4.33	1.95 ± 0.19	73.86 ± 9.08
5	3.88 ± 1.02	1.04 ± 0.21	52.68 ± 14.73	2.13 ± 0.43	66.36 ± 4.19
6	3.06 ± 0.62	1.26 ± 0.11	59.61 ± 11.21	2.04 ± 0.67	75.86 ± 15.37
Mean	3.49 ± 0.92	1.15 ± 0.15	57.32 ± 10.65	1.87 ± 0.31	71.55 ± 9.15

Figure 4-1 illustrates the correlation between rhodanese activity and total antioxidant levels in the seminal plasma, showing a positive trend, further indicating the enzyme's role in maintaining sperm quality through antioxidant mechanisms.

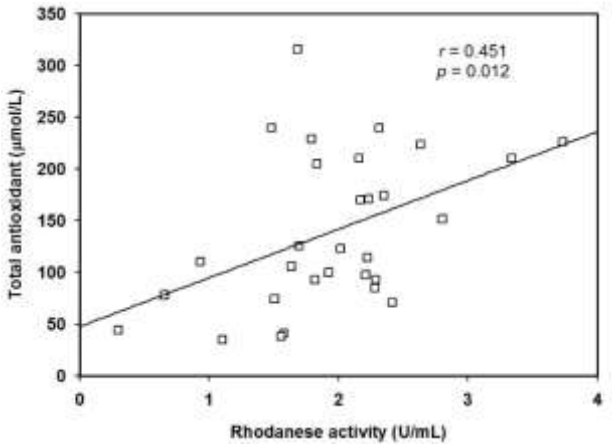

Figure 4-1: Correlation between Rhodanese Enzyme Activity in Seminal Plasma and Total Antioxidant Capacity.

4.2 Relationship Between Rhodanese and Oxidative Stress

- **Levels of Lipid Peroxidation and DNA Fragmentation in Relation to Rhodanese Activity:** The analysis of lipid peroxidation, measured by malondialdehyde (MDA) levels, showed a **moderate negative correlation** with rhodanese activity (r = -0.462, p = 0.01). This indicates that higher rhodanese activity may contribute to reduced lipid peroxidation, suggesting its protective role against oxidative damage to sperm.

In addition, **DNA fragmentation** was assessed, showing a **negative correlation** between rhodanese activity and DNA fragmentation (r = -0.407, p = 0.025). The data suggests that higher rhodanese activity correlates with lower DNA fragmentation in sperm, indicating its potential protective effect on sperm integrity.

- **ROS and Total Antioxidant Capacity as Mediators:** Rhodanese activity also correlated positively with total antioxidant capacity (r = 0.451, p = 0.012), indicating its involvement in enhancing the antioxidant defenses of sperm. Additionally, the negative correlation between rhodanese activity and lipid peroxidation (r = -0.462, p = 0.01) supports the role of rhodanese in mitigating oxidative stress.

Figure 4-2 shows the relationship between rhodanese activity and lipid peroxidation (MDA levels), highlighting the enzyme's role in reducing oxidative damage to sperm. **Figure 4-3** further supports the connection between rhodanese activity and DNA fragmentation, reinforcing the potential protective function of rhodanese in maintaining sperm DNA integrity.

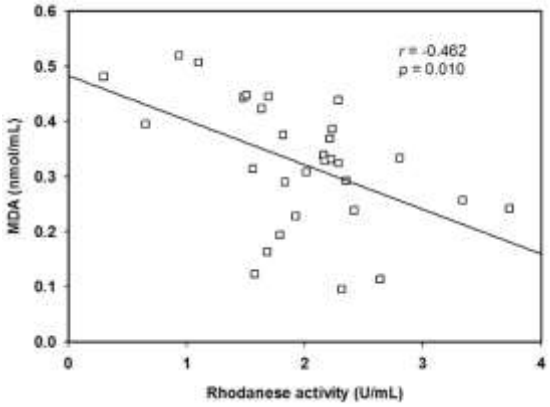

Figure 4-2: Correlation between Rhodanese Enzyme Activity in Seminal Plasma and Lipid Peroxidation.

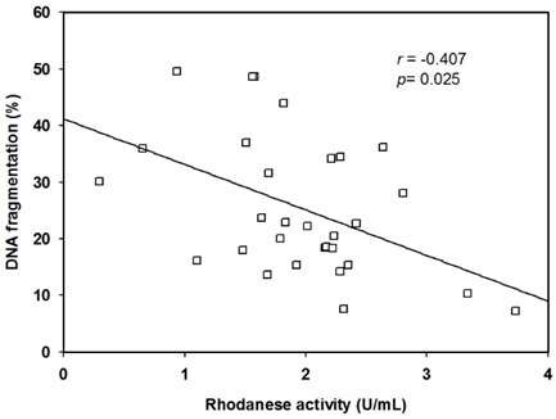

Figure 4-3: Correlation between Rhodanese Enzyme Activity in Seminal Plasma and DNA Fragmentation.

4.3 Comparative Analysis

- **Differences in Outcomes Between Normal and Reduced Rhodanese Activity Groups:** A comparative analysis between groups with normal and reduced rhodanese activity revealed significant differences in sperm quality and oxidative stress markers. The group with **higher rhodanese activity** showed improved sperm motility, higher viability, and reduced sperm abnormalities. Additionally, lower lipid peroxidation levels and less DNA fragmentation were observed in these groups, suggesting the importance of rhodanese in maintaining sperm quality.

In contrast, the **low rhodanese activity** group exhibited higher levels of lipid peroxidation and DNA fragmentation, correlating with poorer sperm quality parameters. This highlights the potential impact of rhodanese activity on sperm quality and oxidative stress regulation, emphasizing its role in male fertility.

The significant findings suggest that **rhodanese activity** could serve as a marker for oxidative stress and sperm quality, potentially guiding therapeutic approaches to improving male fertility.

5. Discussion

5.1 Interpretation of Findings

This study sheds light on the protective role of rhodanese enzyme against oxidative damage in sperm. The data demonstrated significant positive correlations between rhodanese activity and total antioxidant capacity in seminal plasma, indicating its role in enhancing antioxidant defenses. Additionally, a negative correlation between rhodanese activity and both lipid peroxidation and DNA fragmentation underscores its protective mechanism. These findings suggest that rhodanese may play a pivotal role in mitigating oxidative stress, thereby preserving sperm quality and function. This aligns

with the broader understanding that oxidative damage is a major contributor to male infertility, as it compromises key sperm parameters such as motility, viability, and genetic integrity.

5.2 Comparison with Existing Literature

The results of this study align with prior findings on the role of oxidative stress in sperm dysfunction and the importance of antioxidant defenses. Studies by **Mahfouz et al. (2010)** and **Lewis et al. (1995)** have highlighted the detrimental effects of elevated ROS on sperm DNA integrity and motility, emphasizing the need for robust antioxidant systems. Furthermore, the observed reduction in lipid peroxidation with increased rhodanese activity is consistent with findings by **Colagar et al. (2013)** regarding the importance of antioxidants in maintaining sperm membrane integrity. However, this research uniquely highlights rhodanese as a potential enzymatic player in antioxidant defenses within the seminal plasma, a novel contribution to the field.

5.3 Implications for Therapeutics

The findings open new avenues for therapeutic interventions in male infertility. Enhancing rhodanese activity, either through pharmacological agents or dietary supplements, could serve as a strategy to reduce oxidative damage in sperm. Additionally, combining rhodanese-boosting therapies with conventional antioxidants like glutathione or vitamin E could amplify protective effects. This approach holds promise, particularly for individuals with idiopathic infertility where oxidative stress is suspected as a contributing factor.

5.4 Limitations of the Study

While the study provides compelling evidence of the role of rhodanese in reducing oxidative stress, several limitations should be acknowledged:

- **Sample Size:** The study involved a limited number of samples, which may impact the generalizability of the findings.
- **Mechanistic Insights:** Although correlations were established, the exact biochemical pathways through which rhodanese exerts its effects remain to be elucidated.
- **Species-Specific Findings:** The research was conducted on rams, and extrapolation of findings to human fertility may require additional studies.
- **Broader Antioxidant Networks:** Other antioxidant enzymes and their interactions with rhodanese were not explored, which could provide a more comprehensive understanding of its role.

Future research should address these limitations by including larger, diverse sample populations, conducting mechanistic studies, and exploring rhodanese interactions within the broader antioxidant network.

6. Conclusion

Summary of Key Findings

This study underscores the crucial role of rhodanese in modulating oxidative stress and preserving sperm quality. Positive correlations between rhodanese activity and total antioxidant capacity, as well as negative associations with lipid peroxidation and DNA fragmentation, highlight its antioxidant-like functions within seminal plasma. These findings provide novel insights into the mechanisms by which rhodanese may mitigate oxidative damage and improve reproductive health.

Emphasis on Therapeutic Potential

The therapeutic potential of rhodanese lies in its ability to bolster antioxidant defenses and reduce oxidative stress, a key factor in male infertility. Targeting rhodanese through pharmacological or dietary interventions could emerge as a promising strategy for enhancing sperm function and addressing oxidative stress-related infertility.

Recommendations for Future Research

1. **Investigate Rhodanese Functions in Other Reproductive Contexts**
    - Extend studies to assess rhodanese activity in other reproductive tissues and its role in gametogenesis, fertilization, and embryogenesis.
    - Explore its function across different species, including humans, to confirm translational relevance.
2. **Longitudinal Studies on Therapeutic Interventions**
    - Conduct clinical trials to evaluate the efficacy of rhodanese-boosting agents or dietary supplements in improving male fertility outcomes.

- Study the long-term effects of enhanced rhodanese activity on overall reproductive health and offspring quality.

3. **Explore Synergies Between Rhodanese and Other Antioxidants**
    - Investigate how rhodanese interacts with established antioxidant systems, such as glutathione peroxidase and superoxide dismutase.
    - Evaluate combination therapies incorporating rhodanese activators and exogenous antioxidants to maximize therapeutic benefits.

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