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College of Medicine

"Effect of Glutathione on Male Infertility"

A Thesis

Submitted to the Council of the College of Medicine, University of Diyala , Department of Gynecology and Microbiology, as a Partial Fulfillment in the Requirements for the Degree of MBChB

This graduation Research done by:

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Dedication

This work is dedicated to my beloved family, whose unwavering support, encouragement, and love have been my greatest source of strength. To my parents, for their endless sacrifices and guidance, and to my mentors, for inspiring me to achieve my goals. This is for all of you who believed in me.

Acknowledgment

I extend my sincere appreciation to Dr. Mustafa Ahmed Dawood for his unwavering support, expert guidance, and constructive feedback. His valuable insights and encouragement have significantly contributed to the completion of this work. I am truly grateful for his mentorship and the time he dedicated to this study.

SUPERVISOR CERTIFICATIONS

I certify that the preparation of this research titled (Effect of Glutathione on Male Infertility) and conducted by the students (Zahraa Abbas Kadhim) was conducted under my supervision, College of Medicine, Diyala University, which is part of the requirements for obtaining a bachelor's degree in medicine.

Name of supervisor: Dr. Mustafa Ahmed Dawood

Academic degree: Assistant Lecturer

Signature:

Date:

Certificate

We, the examining committee after reading this research and examining the students in its content, find it adequate as a project for the degree of MBChB.

Signature:

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Member:

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List of contents

Contents	Page
Dedication	I
Acknowledgments	II
Certificate	III
Certificate	IV
Contents	V
List of tables	VI
List of figures	VI
Abstract	VII
Chapter One	
Introduction	1
Aim of the study	3
Chapter Two	
Method	4
Chapter Three	
Result	7
Chapter Four	
Discussion	13
Chapter Five	
Conclusion	15
Recommendation	15
References	17

List of Tables

Table No.	Title	Page
1	**Table 1:** Semen Parameters Before and After Glutathione Therapy	8
2	**Table 2:** Statistical Comparison of Semen Parameters Pre- and Post-Treatment	11

List of Figure

Table No.	Title	Page
1	Figure 1: Total count	9
2	Figure 2 : Motility	9
3	Figure 3 : Active	9
4	Figure 4 : Sluggish	9
5	Figure 5 : Morphology	9
6	Figure 6: total count	12
7	Figure 7: motality	12
8	Figure 8: active	12
9	Figure 9: sluggish	12
10	Figure 10: morphology	12

List of Abbrevations

Abbrevations	Meaning
OS	Oxidative stress
ROS	reactive oxygen species
SOD	Superoxide dismutase
GST	glutathione S-transferase (GST) glutathione S-transferase
GPX	glutathione peroxidase

Abstract

Background : Infertility, which is defined as a couple's inability to conceive after a year of trying with frequent, unprotected sexual activity, is one of the most prevalent and significant conditions that afflict an estimated 70 million individuals worldwide. Men are thought to be responsible for 30–50% of infertility reasons, with sperm abnormalities being the most frequently identified cause .

Objective : To evaluate the effectiveness of glutathione therapy in improving sperm parameters specifically motility, total count, morphology, in infertile males.

Method and patient: A retrospective cohort study evaluated the effects of glutathione TAD® therapy (600 mg/4 ml injections) on sperm parameters in 12 infertile men aged 20 to 45 years with idiopathic infertility and normal hormonal profiles. Excluding those with structural, genetic, or chronic diseases, baseline sperm data were collected before participants underwent a 2-month intervention with daily injections. Post-treatment sperm analysis was performed to assess changes in count, motility, and morphology using WHO-compliant kits and a light microscope. Ethical approval was obtained and paired t-tests in SPSS (v26) demonstrated statistically significant ($p<0.05$) improvements in sperm parameters.

Result : The results of the study showed significant improvements in all sperm parameters assessed after glutathione therapy. The mean total sperm count increased significantly from 39 million (± 8.27 million) before treatment to 73.75 million (± 8.06 million) after treatment ($t = -17.92$, $p = 1.72$). The mean motility percentage showed significant improvement increasing from 14.83% (± 7.27%) to 67.83% (± 11.09%) ($t = -15.82$, $p = 6.51$). Similarly, there was a significant increase in the percentage of active sperm from 9.08% (± 5.30%) to 55.00% (± 10.63%) ($t = -22.12$, $p = 1.81$), suggesting an increase in sperm vitality. Although the reduction in slow sperm was modest, with percentages ranging from 5.75% (±5.33%) to 12.83% (±6.66%), it was statistically significant ($t = -2.50$, $p = 0.029$). Additionally, percentage of sperm

with normal morphology significantly increased from 50.83% (± 14.28%) to 63.58% (± 15.84%) (t = -2. 37, p = 0.037).

Conclusion: The study concluded that glutathione therapy significantly improved sperm parameters in infertile men diagnosed with idiopathic infertility.

Chapter One

((Introduction))

Introductions

Infertility – inability of a couple to conceive even after a year of efforts with regular, unprotected sexual activity – is one of the most common and serious issues affecting an estimated 70 million persons worldwide . Sperm abnormalities being the most commonly identified cause of infertility , accounting for 30-50% of the causes believed to be attributable to men, [1].

although there a variety of factors may result in Male infertility which including lifestyle choices, genetic mutations, medical conditions, and drugs. Idiopathic sperm abnormalities remain responsible for about 30% of male infertility[2]. In fact, causes of male infertility and the sperm cell structural and biochemistry defects that impair fertilising capacity are still poorly understood. Abnormal semen quality (primarily motility, morphology, and total count) in male partners from infertile couples shown through various recent studies can serve as a diagnostic marker to identify the infertility reasons [3].

Oxidative stress (OS), which refers to an imbalance between reactive oxygen species (ROS) and antioxidants which may be free radicals or non-radicals in their structure, is an important instrumental in male infertility. There are three identifible major groups of reactive species in biology and medicine studies which are Reactive nitrogen species, reactive chlorine species and reactive oxygen species(ROS). [4] Small amounts of ROS are critical for physiological aspects of sperm such as capacitation, hyperactivation and acrosomal response, as well as the antioxidant shields of the body [2]. Nevertheless, elevated levels of ROS induce infertility in various ways such as lipid peroxidation, DNA, protein oxidation, and enzyme inactivation in spermatozoa. Superoxide dismutase (SOD) and the glutathione peroxidase/reductase pair are two putative forms of defence against superoxide (O2.-) and H2O2, it has been shown in a number of recent studies that spermatozoa can protect themselves from oxidative damage [5].

While not every person with male infertility may experience the same look or level of severity, there are a range of symptoms that can indicate male infertility. Some others may experience testicular pain and swelling, some have fluctuating sexual drives but closely related to fertility health. Additionally, men with fertility problems often have problems with erections[6]. because OS is a consequence of oxidative damage, lifestyle changes and antioxidant therapy are effective, in combination with identifying the main infertility cause as well as excluding other causes such as erectile dysfunction or hormonal insufficiency, in order to have a sperm quality and reproductive outcomes ameliorated [7].

Glutathione, a tripeptide thiol—a non-protein sulfhydryl compound—found naturally in mammalian cells is the endogenous antioxidant. It is composed of three amino acids, namely glutamate, cysteine, and glycine. is present in somatic cells and germ cells, male and female gametes, very important in detoxifying reactive oxidants. There are two forms of glutathione: GSH and GSSG. Reduced levels of glutathione in males lead to impaired motility and produce a protective coating to protect spermatozoa plasma membrane [8].

Glutathione (L-γ-glutamyl-L-cysteinylglycine; GSH), the most abundant non-protein thiol in mammalian cells, is present at concentrations between 0.5–10 mmol/L. Cellular GSH is important for multiple biological functions such as protein and DNA synthesis, amino acid transport and, most importantly, guarding against oxidative damage. Glutathione serves as an antioxidant due to its sulphydryl (SH) group, which is a powerful nucleophile that can protect cellular components from oxidation from oxidants, electrophiles, and free radicals.

STF cells are the producers of GSH and may play a role in the synthesis of GSH in germ cells. Although the testis, reproductive tract fluids, and epididymal sperm contain large amounts of GSH, the levels in ejaculated spermatozoa are much lower. Glutathione participates in protective mechanisms, specifically the detoxification of

reactive oxygen species (ROS) and peroxides generated in the oxidation of oxygen by cells.

It's essential to note that the main function of glutathione in the reproductive system is preventive against ROS. Finally, this shows its crucial role in preserving the biological properties of germ cells and its participation in fertilization and early embryogenesis [9].

The antioxidant action of intracellular GSH improves sperm morphology and motility in adult males according to research. Antioxidant-associated genes, such as glutathione S-transferase (GST) and glutathione peroxidase (GPX) are essential for modulation of sperm functionality and spermatogenesis. Significantly increase the risk of subfertility, oligozoospermia, oligoasthenoteratozoospermia and decrease of overall sperm quality[10].

It is thought that antioxidant supplementation can enhance sperm quality by decreasing oxidative stress, quenching free radicals, and promoting the health of sperm cells. That supplementation has been shown to improve sperm motility, concentration, and the structural integrity of the sperm. It also minimizes oxidative damage to sperm DNA, which can enhance fertilization success rates and reduce the potential for genetic abnormalities in offspring [11].

Aim of study : To evaluate the effectiveness of glutathione therapy in improving sperm parameters specifically motility, total count, morphology, in infertile males.

Chapter Two

((Patients and Methods))

Method and Materials

1.Study Design : A retrospective cohort study was conducted to evaluate the effects of glutathione therapy on sperm parameters in infertile males

Data were obtained from medical and laboratory records of patients treated at a reproductive health clinic using TAD® 600 mg/4 ml glutathione injections.

2.Study Population : A total of 12 male participants.

Inclusion Criteria:

• Male patients aged 20–45 years.

• Diagnosed with infertility for more than one year.

• Normal hormonal profiles (e.g., testosterone)

• History of idiopathic infertility and dyspermia.

Exclusion Criteria:

• Structural or genetic abnormalities causing infertility.

• Chronic illnesses such as diabetes or cardiovascular diseases.

• Recent use of medications affecting fertility.

3.Materials

.Reagents and Equipment:

• TAD® Glutathione Injection Kit: Manufactured by Biomedica Foscama, Italy (600 mg powder for reconstitution with solvent(

• Light Microscope: Manufactured by Leica Microsystems, used for sperm motility and morphology analysis.

• Slides and Cover Slips: Provided by Thermo Fisher Scientific.

• Incubator: Manufactured by Binder GmbH, Germany, maintained at 37°C for sample processing.

• Semen Analysis Kits: WHO-compliant, sourced from Hamilton Thorne, Inc. for sperm evaluation.

4.Methods

1. Baseline Data Collection:

Medical records were reviewed to retrieve pre-treatment data, including semen parameters (count, motility, and morphology) .

2. Intervention:

Participants received glutathione injections (600 mg intramuscularly) every other day for a period of 2 months.

The reconstitution of TAD® powder followed the manufacturer's instructions provided by Biomedica Foscama.

3. Post-Treatment Assessment:

Follow-up data on semen analysis and oxidative stress markers were collected after 2 months of therapy.

Semen smears were examined under a light microscope to evaluate motility, count, and morphological changes.

4. Incubation Protocol:

All semen samples were incubated at 37°C using an incubator prior to analysis to maintain optimal sample conditions.

5.Ethical Approval : The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of the clinic. We informed consent was obtained from all participants before their medical records were included in the analysis.

6.Statistical Analysis

- Paired t-tests were used to compare pre- and post-treatment sperm parameters and oxidative stress markers.
- Data were expressed as mean ± standard deviation, with a p-value < 0.05 considered statistically significant.
- Statistical analyses were performed using SPSS (26) version.

Chapter Three

((Results))

Results

Table 1: The descriptive analysis revealed significant improvements in all measured semen parameters following glutathione therapy.

1. Total Count: The mean total sperm count increased significantly from 39 million (± 8.27 million) pre-treatment to 73.75 million (± 8.06 million) post-treatment, reflecting a marked enhancement in sperm quantity.
2. Motility: A substantial improvement in motility was observed, with the mean percentage increasing from 14.83% (± 7.27%) to 67.83% (± 11.09%), indicating better sperm movement capabilities.
3. Active Sperm: The proportion of active sperm demonstrated a considerable rise, from 9.08% (± 5.30%) pre-treatment to 55.00% (± 10.63%) post-treatment, suggesting enhanced sperm vitality.
4. Sluggish Sperm: A modest reduction in sluggish sperm was noted, with percentages decreasing from 5.75% (± 5.33%) to 12.83% (± 6.66%).
5. Morphology: The percentage of sperm with normal morphology improved from 50.83% (± 14.28%) to 63.58% (± 15.84%), indicating an enhancement in structural quality.

Table 1: Semen Parameters Before and After Glutathione Therapy

Variable	N	Mean	Std. Deviation
Before			
Total Count	12	39000000.00	8268230.546
Motality	12	14.83%	7.272%
Active	12	9.08%	5.299%
Sluggish	12	5.75%	5.328%
Morphology	12	50.83%	14.282%
After			
Total count	12	73750000.00	8058028.182
Motality	12	67.83%	11.085%
Active	12	55.00%	10.634%
Sluggish	12	12.83%	6.658%
Morphology	12	63.58%	15.843%

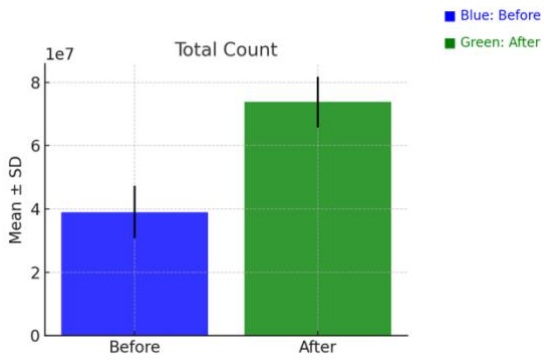


Figure 1: Total count

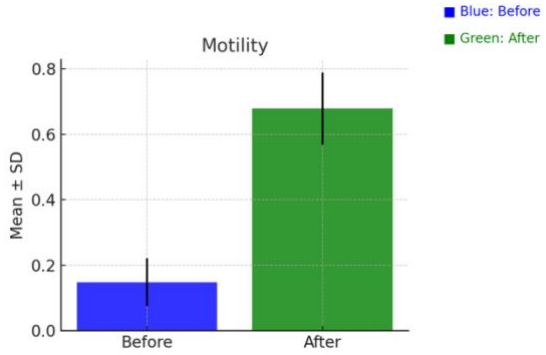


Figure 2 : Motility

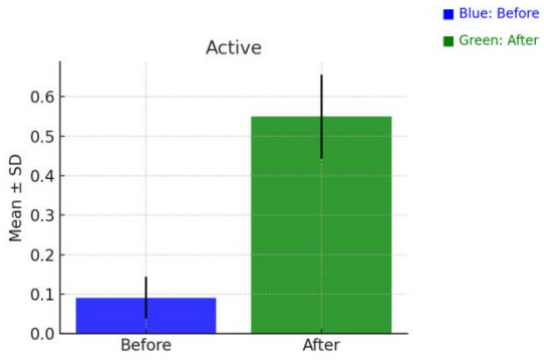


Figure 3 : Active

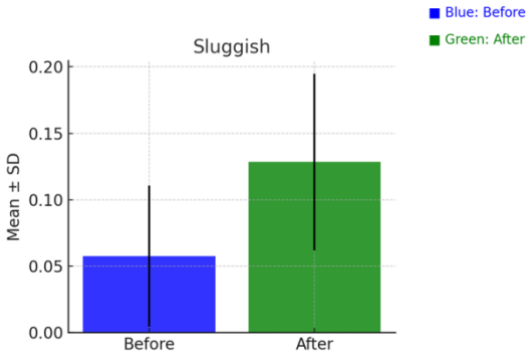


Figure 4 : Sluggish

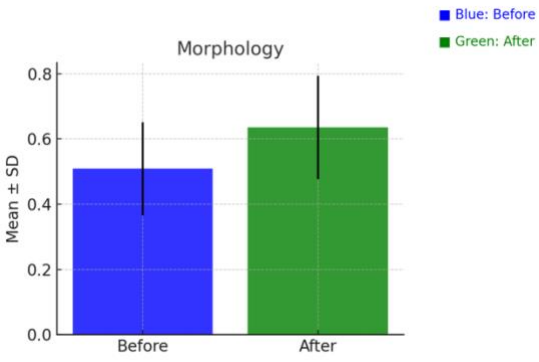


Figure 5 : Morphology

Table 2 : Paired t-tests were performed to evaluate the statistical significance of changes in semen parameters pre- and post-treatment. The analysis confirmed significant improvements across all measured parameters:

1. Total Count: A significant increase was observed, with a t-statistic of -17.92 and a p-value of 1.72×10^{-9}.

2. Motility: The improvement in motility was highly significant, with a t-statistic of -15.82 and a p-value of 6.51×10^{-9}.

3. Active Sperm: The increase in active sperm percentage was statistically significant, with a t-statistic of -22.12 and a p-value of 1.81×10^{-10}.

4. Sluggish Sperm: Although the reduction in sluggish sperm percentage was modest, it remained statistically significant (t-statistic = -2.50, p-value = 0.029).

5. Morphology: The improvement in sperm morphology was significant, with a t-statistic of -2.37 and a p-value of 0.037

Table 2: Statistical Comparison of Semen Parameters Pre- and Post-Treatment

Parameter	t-Statistic	df	sig (2-tailed)	p-Value
Total Count	-17.922	11	1.727	1.727
Motility	-15.817	11	6.510	6.510
Active	-22.117	11	1.8111	1.811
Sluggish	-2.503	11	0.0293	0.029
Morphology	-2.374	11	0.0368	0.036

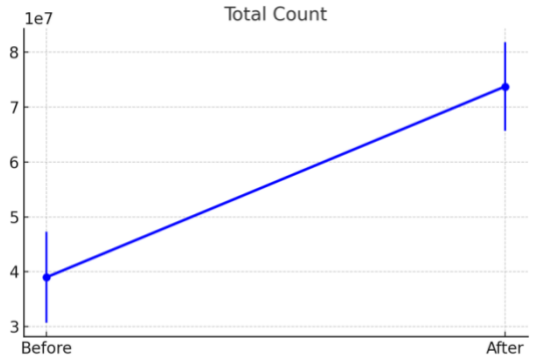


Figure 6: total count

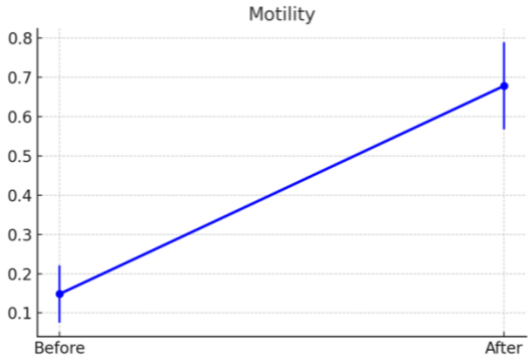


Figure 7: motality

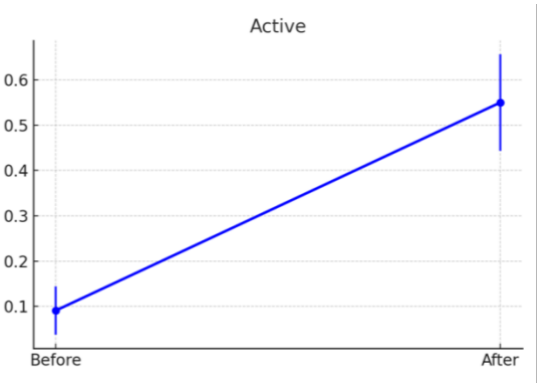


Figure 8: active

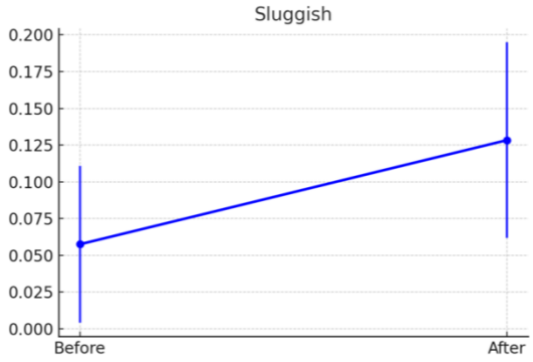


Figure 9: sluggish

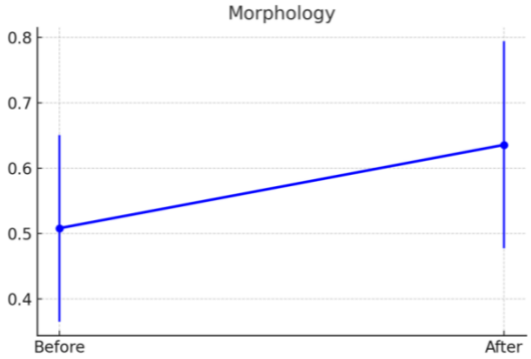


Figure 10: morphology

Chapter Four

((Discussion))

Discussion

Through our study, we note that glutathione treatment led to an improvement in sperm parameters in males diagnosed with unexplained infertility.. In particular total sperm counts increased from 39 ± 8.27 million to 73.75 ± 8.06 million, motility from 14.83% ± 7.27% to 67.83% ± 11.09%, active from 9.08% to 0% ± 0.8% normal. 0 ± 5% normal. morphology improved from (50.83% ± 14.28%) to (63.58 ± 15.84%). Through these results, we notice a significant improvement in sperm quality and activity after treatment with glutathione. Likewise, a study by Lenzi et al. (1993) [12] Significant improvements in sperm parameters were shown after glutathione use in men with secondary sexual dysfunction such as unilateral varicocele or infertile genital tract infection. Their study reported significant improvements in sperm motility and morphology after glutathione use, and suggested that these effects were due to the antioxidant properties of glutathione. In our study, we found that glutathione treatment led to significant improvements in all sperm parameters. There was a significant increase in total sperm count (t- statistic = -17.92 (p = 1.72)), suggesting an improvement in sperm quantity in these men. Likewise, the sperm motility t-statistic was -15.82 (p = 6.51) which basically led to this significant decrease and increase based on the sperm motility. The percentage of active sperm was the parameter with the most significant improvement, t-statistic = -22.12 (p = 1.81 ×), demonstrating the vitality of sperm. A small but statistically significant decrease was seen in lazy sperm with a t-statistic of −2.50 (p = 0.029). Furthermore, there was a significant improvement in sperm morphology with a t-statistic of -2.37 (p = 0.037), indicating a beneficial effect on sperm shape and quality as well. These results are consistent

This is in agreement with the study by Lenzi et al. (1993) [12], Significant improvements in sperm motility and morphology were demonstrated after glutathione use in males with dyszoospermia caused by conditions related to oxidative stress, such as unilateral varicocele or genital tract inflammation. Linzi and colleagues suggested that these improvements were due to the antioxidant properties of glutathione, which effectively reduced oxidative stress and improve sperm function

Chapter Five

((Conclusion and Recommendations))

Conclusion

The results of this study indicate that glutathione treatment improved sperm parameters in men diagnosed with unexplained infertility. We notice a clear increase in the total sperm count, motility and vitality levels, especially those with normal shape, after using the treatment. These results are evidence of the significant role of glutathione in reducing oxidative stress, which is one of the most important causes of unexplained male infertility, and which has a role in improving sperm quality and function in general. These results were obtained by collecting statistically significant parameters that indicate the effectiveness of glutathione in treating dysspermia and obtaining better fertility results in these patients.

Recommendation

1. **Guatathione Therapy:** Glutathione is used to treat male infertility. The most important cases that benefit from it are those due to oxidative stress and poor sperm parameter
2. **Administration:** To obtain faster results and better bioavailability, it is preferable to give the treatment intramuscularly.
3. **Combination therapy:** to enhance the effectiveness of glutathione is recommended to be combined with other antioxidants, for example coenzyme Q10.
4. **Long-term studies:** are being conducted to test the sustainability of the effects of glutathione on fertility and live births.

5. **Patient populations:** To have the potential to improve the opacity, consistency and robustness of results, larger and more diverse patient groups must be included.
6. **Standardized protocols:** Standardized treatment protocols for the use of the treatment with fixed recommendations for the indicated dosage and method of administration.
7. **Intended use:** Use of baseline sperm parameters and oxidative stress markers to administer treatment to patients who need it.

Appendix

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الخلاصة

تخلص الدراسة إلى أن العلاج بالجلوتاثيون يعزز بشكل كبير من معايير السائل المنوي لدى الرجال المصابين بالعقم غير المبرر. أظهر العلاج زيادة ملحوظة في العدد الإجمالي للحيوانات المنوية، وحركتها، وحيويتها، بالإضافة إلى تحسين نسبة الحيوانات المنوية ذات الشكل الطبيعي. تشير هذه النتائج إلى أن الجلوتاثيون يقلل بشكل فعال من الإجهاد التأكسدي، وهو عامل رئيسي في العقم الذكري، مما يحسن جودة الحيوانات المنوية ووظيفتها بشكل عام. تؤكد النتائج ذات الدلالة الإحصائية عبر جميع المعايير المقاسة على إمكانات الجلوتاثيون كعلاج واعد للتعامل مع اضطرابات الحيوانات المنوية وتحسين نتائج الخصوبة في هذه الفئة.



جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ديالى

كلية الطب



"تأثير الجلوتاثيون على العقم الذكري"

أطروحة

مقدمة إلى مجلس كلية الطب، جامعة ديالى، قسم النسائية والتوليد والأحياء الدقيقة، كجزء من متطلبات نيل درجة البكالوريوس في الطب والجراحة العامة(MBChB)

هذا البحث مقدم من قبل:

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بإشراف:

الدكتور مصطفى أحمد داوود

2025