RESEARCH ARTICLE

F.U. Vet. J. Health Sci.
2026; 40 (1): 09 - 13
http://www.fusabil.org

Effect of Adding Different Amounts of *Ganoderma Lucidum* (Reishi) to Sperm Extender on the Freezing of Ram Sperm [*]

Şeyma ÖZER KAYA [1, a]
Tutku Can ACISU [1, b]
İbrahim Halil GÜNGÖR [1, c]
Emre KAYA [2, d]
Aslıhan ÇAKIR CİHANGİROĞLU [3, e]
Seyfettin GÜR [1, f]
Mustafa SÖNMEZ [1, g]
Gaffari TÜRK [1, h]

[1] Fırat University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Elazığ, TÜRKİYE

[2] Fırat University, Faculty of Veterinary Medicine, Department of Biochemistry, Elazığ, TÜRKİYE

[3] Siirt University, Faculty of Veterinary Medicine, Department of Department of Reproduction and Artificial Insemination, Siirt, TÜRKİYE

[a] ORCID: 0000-0002-9970-9364
[b] ORCID: 0000-0002-0882-937X
[c] ORCID: 0000-0002-5250-1478
[d] ORCID: 0000-0002-7445-3091
[e] ORCID: 0000-0003-3365-8960
[f] ORCID: 0000-0003-0096-2501
[g] ORCID: 0000-0003-0281-7228
[h] ORCID: 0000-0001-7417-1038

Received : 15.10.2025
Accepted : 19.12.2025

Correspondence

Şeyma ÖZER KAYA
Fırat University,
Faculty of Veterinary Medicine,
Department of Reproduction and Artificial Insemination
Elazığ – TÜRKİYE

sozer@firat.edu.tr

This research aimed to improve the post-thaw quality of ram semen by reducing oxidative stress during the freezing process through the use of *Ganoderma lucidum* (Reishi), a mushroom known for its potent antioxidant properties. Semen samples collected from 6 Akkaraman rams were pooled and divided equally into control and treatment groups. The study consists of six groups: Group 1 (Control, 0% GL), Group 2 (0.5% GL), Group 3 (1% GL), Group 4 (2% GL), Group 5 (4% GL), Group 6 (8% GL). After dilution and cooling, the semen was glycerolized, equilibrated, packaged into 0.25 ml mini straws, and cryopreserved using an automated freezing system. Results showed that supplementation with 0.5%, 1%, 2% GL significantly improved total and progressive motility compared with the control group. In contrast, the 8% GL treatment exhibited toxic effects on sperm, leading to reduced motility. In the kinematic analysis, significant changes were detected in velocity of curvilinear and velocity of average path values of 8% GL compared to all other groups. In the oxidative stress analysis, malondialdehyde concentrations were significantly higher in the 8% GL group compared with the control group. Glutathione concentrations and catalase activity increased in the 1% and 2% GL groups and decreased in the 8% GL group. Glutathione peroxidase activity increased in the 0.5%, 1% and 2% groups and decreased in 8%. Superoxide dismutase activity decreased in the 8% GL group but remained unchanged in the other treatment groups. Overall, the findings suggest that supplementation of extenders with 0.5%, 1%, or 2% GL extract enhances semen cryopreservation quality in rams.

Key Words: *Ram, sperm, Ganoderma lucidum, cryopreservation, oxidative stress*

Sperma Sulandırıcısına Farklı Oranlarda Ganoderma Lucidum (Reishi) İlavesinin Koç Spermasının Dondurulması Üzerine Etkisi

Bu araştırma, güçlü antioksidan özellikleriyle bilinen bir mantar olan *Ganoderma lucidum* (Reishi) kullanılarak dondurma işlemi sırasında oluşan oksidatif stresi azaltarak koç semeninin çözülme sonrası kalitesini iyileştirmeyi amaçlamaktadır. Altı Akkaraman koçundan toplanan semen örnekleri bir araya getirilerek kontrol ve tedavi gruplarına eşit olarak bölünmüştür. Çalışma altı gruptan oluşmaktadır: Grup 1 (Kontrol, %0 GL), Grup 2 (%0.5 GL), Grup 3 (%1 GL), Grup 4 (%2 GL), Grup 5 (%4 GL) ve Grup 6 (%8 GL). Seyreltme ve soğutma işleminin ardından semen gliserolize edilmiş, dengelenmiş, 0.25 ml'lik mini payetlere paketlenmiş ve otomatik dondurma sistemi kullanılarak kriyoprezervasyona tabi tutulmuştur. Sonuçlar, %0.5, %1 ve %2 GL takviyesinin, kontrol grubuna kıyasla toplam ve ilerleyici hareketliliği önemli ölçüde iyileştirdiğini göstermiştir. Buna karşılık, %8 GL tedavisi sperm üzerinde toksik etkiler göstererek hareketliliğin azalmasına yol açmıştır. Kinematik analizde, %8 GL diğer tüm gruplara göre eğrisel hız ve ortalama yol hızı değerlerinde önemli değişiklikler saptanmıştır. Oksidatif stres analizinde, kontrol grubuyla karşılaştırıldığında, malondialdehit konsantrasyonları %8 GL grubunda önemli ölçüde yüksektir. Glutatyon konsantrasyonları ve katalaz aktivitesi %1 ve %2 GL gruplarında artmış, %8 GL grubunda azalmıştır. Glutatyon peroksidaz aktivitesi %0,5, %1 ve %2 gruplarında artmış, %8'de azalmıştır. Süperoksit dismutaz aktivitesi %8 GL grubunda azalmış ancak diğer tedavi gruplarında değişmemiştir. Bulgular genel olarak, koçlarda semen kriyoprezervasyonunun kalitesinin %0.5, %1 veya %2 GL ekstresi ile desteklenmesiyle arttığını göstermektedir.

Anahtar Kelimeler: *Koç, sperm, Ganoderma lucidum, dondurarak saklama, oksidatif stres*

Introduction

Despite numerous studies, the freezing of semen in rams has not yet been succesfull. The different lipid structure of the sperm of this species compared to other species is thought to be the main reason for this problem. The freezing process creates various physical and chemical stresses in ram semen, which negatively impacts the success of this process (1).

Numerous antioxidants have been added to sperm extenders to reduce the damage caused by these stresses on spermatozoa and to maintain their quality during freezing, but the desired success has not yet been achieved (2-4).

The temperature changes that occur during the freezing-thawing processes cause physical and chemical stress in mammalian spermatozoa, leading to changes in the lipid composition of the plasma membrane (5, 6). It is suggested that the damage occurring

[*] This study was supported by the Firat University Scientific Research Project Unit with project number VF.22.21.

during freezing of ram semen is due to peroxidation of membrane lipids induced by increased oxidative stress levels due to ice crystal formation (7). It has been reported that freezing and thawing causes many damage in ram sperm, including loss of motility, increased rates of dead and abnormal spermatozoa, spermatozoa with damaged plasma and acrosomal membranes, and increased LPO, an indicator of disruptions in the oxidant-antioxidant balance (8, 9).

Ganoderma lucidum (GL) is a mushroom widely used in Chinese medicine that contains biologically active compounds, including polysaccharides, phenols, proteins, polysaccharide-protein complexes, lipid components, and terpenoids (10). GL has been reported to have antioxidant (11), and anti-inflammatory effects (12).

GL has also been reported to have a protective effect against disorders associated with the male genital system (13). Another study reported that GL protects testicular histology, improves sperm quality, enhances antioxidant status, and prevents apoptosis (14). Tutku et al. (15) found that in rats exposed to the endocrine-disrupting chemical bisphenol A, the GL treatment group provided protection against disrupted oxidant balance (p<0.001). Most of these studies indicate that Reishi extract can positively affect sperm quality and production due to its antioxidant capacity.

Scientific research has not yet directly examined the effects of Ganoderma lucidum (Reishi mushroom) on ram semen. There has been no study yet investigating how spermatological values change during freezing and thawing of ram semen and its effects on oxidative stress. However, the effects of the GL mushroom on male reproductive health have been extensively studied, particularly in experimental studies conducted on rats, and the positive results obtained in rats demonstrate its potential benefits for male reproductive health. This study aimed to determine the effects of adding different amounts of Ganoderma lucidum extract to a semen extender on antioxidant properties and cryopreservation quality in ram semen.

Materials and Methods

Research and Publication Ethics: Approval was obtained from the Firat University Animal Experiments Local Ethics Committee dated 11.05.2022, with session number 2022/08.

Animal Material: Six 2-year-old Akkaraman rams, clinically healthy, with no pathological findings during genital examination, and weighing an average of 60-65 kg, were used. Before and throughout the experimental studies, the rams were fed high-quality forage and concentrates. Drinking water was provided ad libitum. Semen samples were collected from 6 healthy Akkaraman rams by artificial vagina 3 times for two weeks (3 replications).

Experimental Design and Formation of Study Groups: For this purpose, a stock solution (SS) of GL liquid extract (Immunat, herbal, Turkey) was obtained. Stock solutions were diluted again with Tris+egg yolk extender (TYSS) [20 mL egg yolks + 80 mL Tris diluent (Tris hydroxymethylene aminomethane 3.63 g, fructose 0.50 g, citric acid 1.99 g] and in the final solution, GL was adjusted to 0.5%, 1%, 2%, 4%, and 8% before 5°C. Sperm were collected from rams using an artificial vagina. Motility of the sperm brought to the laboratory were determined using a computer-aided sperm analysis (CASA-ISASv1, Proiser, Spain). For the collected sperm to be used in the study, total motility was required to be above 70% and density above 3 billion. Sperm meeting these requirements were diluted 1:1 with previously prepared TYSS. The diluted sperm were pooled. The pooled samples were divided into 2 ml aliquots for each study group and combined with the study groups. In this way, experimental groups were created [Group 1: 0% GL (Control), Group 2: 0.5% GL, Group 3: 1% GL, Group 4: 2% GL, Group 5: 4% GL, and Group 6: 8% GL]. The temperature of the diluted semen was then lowered to 5°C in the cooling cabinet, and the experimental groups were gradually combined with 10% glycerol TYSS containing the same proportions of GL. Following this process, the semen was kept in the cooling cabinet for 4 hours and then drawn into 0.25 mL mini straws. They were frozen in an automatic freezing device (Microdigitcool, IMV, France). Frozen semen was stored in containers containing liquid nitrogen at -196°C. Twenty-four hours after freezing, the straws were thawed in 25 seconds in water at 38°C. Oxidative stress parameter levels were measured in thawed semen using spermatological analyses.

Spermatological Analyses

Motility and Kinematic Parameter Analysis: At this stage, motility and kinematic parameters were determined in sperm using the Computer-Assisted Sperm Analysis (CASA) system. Thawed semen was diluted 1:4 with Tris buffer, and 3 µL was placed on a special slide (Spermtrack 20 µm). Total, progressive motility rates (%), kinematic parameter values were recorded through the motility module.

Oxidative Stress Analyses: Frozen ram sperm samples were thawed at room temperature. All samples were then centrifuged at 600 g x 10 minutes x 4°C to remove the semen extender, which was added to increase sperm volume, preserve sperm viability, protect sperm from sudden temperature changes, and prevent microorganisms from damaging the sperm. The cellular pellet was washed three times with distilled water and centrifuged each time to preserve the pellet (600 g x 10 minutes x 4°C). The cell pellet was then separated, weighed, and diluted with distilled water at a 1:10 (weight/volume) ratio. All samples were then homogenized with a Bullet Blender Tissue Homogenizer at speed 10 for 4 minutes. A homogenization bead (0.9-2.0 mm stainless steel bead (SSB14B)) was used in the same ratio as the sample volume. The homogenate was centrifuged at 3000 g for 15 minutes at 4°C to determine malondialdehyde (MDA), glutathione (GSH) levels, and catalase (CAT), glutathione-S-transferase (GST) and superoxide dismutase (SOD) activities, and at 10000 g for 55 minutes to determine glutathione peroxidase

(GSH-Px) activity (Hettich Mikro 200R, Tuttlingen, Germany). The supernatant was used for MDA, GSH, CAT, GST, SOD, and GSH-Px analyses (16-21).

Statistical Analyses: Data obtained from the study are presented as the mean ± standard error (SPSS 22 software). Statistical significance was assessed at $p<0.05$, which was considered significant. The Shapiro-Wilk normality test was performed to determine whether all measured parameters were normally distributed, and the results revealed that all parameters were normally distributed. The homogeneity of the variances was evaluated according to the Levene test, and according to the result of the Levene test, it was concluded that there was homogeneity among the variances. Based on these findings, one-way analysis of variance (ANOVA) was used to examine differences between groups, and post hoc Tukey test was used for pairwise comparisons.

Results

Motility and Kinematic Parameters: Among thawed semen, 0.5%, 1%, and 2% GL significantly ($p<0.05$) increased total and progressive motility compared to the control group. 8% GL significantly ($p<0.05$) decreased total and progressive motility compared to the control group (Table 1). Statistical analyses of post-freeze-thaw kinematic parameters revealed statistically significant differences in VCL and VAP values in the 8% GL group compared to the control and other experimental groups (Table 2).

Oxidative Stress Values: Table 3 presents the findings for MDA and GSH levels, along with CAT, GSH-Px, GST, and SOD activities following the freeze–thaw process. In comparison with the control group, MDA concentrations were significantly elevated in the 8% GL group, whereas no meaningful differences were detected in the 0.5%, 1%, 2%, and 4% GL groups. GSH concentrations and CAT activity increased in the 1% and 2% GL groups, decreased in the 8% GL group, and remained unchanged at 0.5% and 4%. GSH-Px activity was enhanced in the 0.5%, 1%, and 2% groups, reduced at 8%, and showed no difference at 4% relative to the control. SOD activity was reduced in the 8% GL group but did not vary in the other treatment groups. GST activity showed no statistically significant variation across any of the groups.

Table 1: Total and progresive motility (%)

Examination Groups	Total motility (%)	Progresive motility (%)
Control	$50.20±6.35^{a}$	$17.80±1.95^{a}$
%0.5 GL	$55.80±6.47^{b}$	$19.80±2.33^{b}$
%1 GL	$57.20±7.34^{b}$	$19.00±2.62^{b}$
%2 GL	$55.60±5.60^{b}$	$18.40±1.20^{b}$
%4 GL	$49.20±10.54^{ab}$	$15.60±3.31^{ab}$
%8 GL	$22.80±3.72^{c}$	$6.80±1.31^{c}$

a, b and c: The difference between values expressed with different letters in the same column is statistically significant ($p<0.05$)

Table 2. Kinematic parameters

Kinematic Parameters	Examination Groups					
	Control	%0.5 GL	%1 GL	%2 GL	%4 GL	%8 GL
VCL	$106.32±3.51^{a}$	$105.76±3.62^{a}$	$107.60±7.84^{a}$	$101.90±3.09^{a}$	$106.44±7.12^{a}$	$76.48±6.58^{b}$
VSL	54.60±3.55	55.60±4.03	56.00±5.74	51.60±3.95	54.80±1.68	40.20±3.83
VAP	$71.60±3.72^{a}$	$72.00±3.98^{a}$	$74.60±6.51^{a}$	$68.60±3.77^{a}$	$74.00±3.25^{a}$	$51.60±4.48^{b}$
LIN	51.20±3.67	52.40±3.74	51.80±2.63	51.00±4.23	52.00±2.70	52.00±1.51
STR	75.80±1.85	76.80±2.22	74.40±1.02	77.00±2.84	74.00±1.70	76.80±0.96
WOB	67.60±3.66	67.80±3.42	69.20±2.88	65.40±3.37	69.80±2.47	67.60±1.50
ALH	4.04±0.30	4.04±0.20	4.10±0.29	4.10±0.23	3.94±0.29	2.94±0.29
BCF	7.72±0.14	7.56±0.36	7.94±0.24	7.52±0.32	8.14±0.40	7.22±0.30

a and b: The difference between values expressed with different letters in the same line is statistically significant ($p<0.05$)

Table 3. MDA, GSH levels, CAT, GSH-Px, GST, and SOD activities determined after freezing and thawing in the control and experimental groups (Mean ± standard error)

Groups	MDA (nmol/g tissue)	GSH (µmol/mL)	CAT (k/g prot.)	GSH-Px (U/g prot.)	GST (U/mg prot.)	SOD (U/mg prot.)
Control	$0.49±0.01^{ab}$	$5.36±0.05^{b}$	$15.53±0.22^{b}$	$86.68±1.27^{b}$	38.25±0.22	$0816±0.01^{bc}$
%0.5 GL	$0.48±0.01^{a}$	$5.61±0.13^{b}$	$17.37±0.40^{bc}$	$95.17±1.34^{cd}$	39.01±0.18	$0.793±0.01^{b}$
%1 GL	$0.45±0.01^{a}$	$6.32±0.07^{d}$	$19.29±0.34^{d}$	$96.86±1.50^{cd}$	38.27±0.26	$0.855±0.03^{bc}$
%2 GL	$0.46±0.01^{a}$	$6.10±0.13^{cd}$	$18.16±0.56^{cd}$	$101.24±1.33^{d}$	38.48±0.20	$0.894±0.02^{c}$
%4 GL	$0.53±0.02^{b}$	$5.62±0.11^{bc}$	$16.56±0.64^{bc}$	$92.20±2.07^{bc}$	38.29±0.33	$0.801±0.02^{b}$
%8 GL	$0.65±0.01^{c}$	$4.20±0.16^{a}$	$12.65±0.39^{a}$	$74.71±1.60^{a}$	38.48±0.22	$0.681±0.02^{a}$

a, b, c and d: The difference between values expressed with different letters in the same column is statistically significant ($p<0.05$)

Discussion

A literature search revealed no studies on the addition of GL extract to semen extenders in rams. Büyük and colleagues (22) explored the impact of different doses of GL extract on rat testicular tissue, spermatogenic cells, and sperm motility. In their study, 40 male Wistar albino rats received daily oral gavage of 500 mg/kg, 2500 mg/kg, or 5000 mg/kg GL extract for nine consecutive days. The findings indicated that low and medium doses promoted spermatogenesis, increased total epididymal sperm count, and enhanced progressive sperm motility. In contrast, the highest dose caused slight testicular damage and did not produce a significant improvement in sperm parameters. Based on these results, the researchers suggested avoiding oral administration of doses exceeding 2500 mg/kg. In our study conducted on rams, a toxic dose (8%) was determined to negatively affect sperm parameters when added to a semen extender. In contrast to the damage caused by high levels of GL supplementation in frozen-thawed spermatozoa, this study also demonstrated that low levels (0.5%, 1%, and 2%) had significantly positive effects on total and progressive sperm motility.

Excessive production of oxidant molecules and a deficiency in the antioxidant defense system disrupt the oxidative balance (23). Oxidative stress causes various cellular and DNA damage, and also damages mitochondrial DNA, which lacks histone wrapping and repair mechanisms. Mitochondrial damage leads to increased oxidative stress and decreased ATP levels, which in turn leads to activation of the cell death pathway (24).

The antioxidant properties of GL extract are known, and our study supports this literature. The positive effects observed in spermatology are thought to be due to GL's basic antioxidant properties by reducing free radical production, scavenging superoxide and hydroxyl radicals, and suppressing the production of reactive oxygen species (25).

Acısu et al. (15) investigated the effects of GL on testicular dysfunction in rats exposed to Bisphenol A, an endocrine-disrupting chemical. For this purpose, they administered 25 mg/kg/bw BPA and 300 mg/kg/bw GL to rats via oral gavage for 8 weeks. They found that CAT, GPx activity, and GSH levels were reduced, while the GL treatment group provided protection against disrupted oxidant balance ($p<0.001$).

Yi et al. (26) investigated the optimal concentration of GLP added to semen cryopreservation extenders at different concentrations after thawing, finding a GLP concentration of 0.8 mg/mL. They reported increased viability in goat sperm, significantly preserved plasma membrane integrity and mitochondrial activity after thawing compared to the control group, and significantly increased antioxidant capacity ($p<0.05$).

Ozmen et al. (27) in their study investigating the effect of electromagnetic fields (EMF) generated by 10 kV (50 Hz) high-voltage power lines on epididymal sperm characteristics and the protective effects of GL and melatonin in male rats exposed to EMF, evaluated epididymal sperm concentration, motility, and morphology. Their study found that EMF exposure could have different effects on sperm quality depending on the duration of exposure; GL treatment increased sperm concentration and reversed the negative effect of EMF on sperm morphology in rats exposed to EMF for 26 or 52 days; and melatonin treatment had negative effects on sperm concentration and sperm morphology in rats exposed to EMF for 26 or 52 days.

Ghajari et al. (28) investigated the protective effect of *Ganoderma lucidum* on mice exposed to the antidepressant drug sertraline. For 35 days, rats receiving SRT were also given GL extract (at a dosage of 300 mg/kg). Furthermore, sperm parameter assessment using CASA results revealed that sperm volume, motility, and viability were significantly lower in the SRT group ($p<0.001$). Administration of GL extract to animals receiving SRT may have reduced histological changes. They found that GL significantly reduced lipid peroxidation in spermatozoa. They also determined that GL protected the testes of mice from damage caused by SRT, likely due to its ability to inhibit reactive oxygen species.

The addition of 0.5%, 1%, and 2% GL extract to semen extenders in rams protected spermatozoa against damage during freezing and thawing and improved freezing quality due to its antioxidant properties. Adding GL extract to ram semen extenders at the specified concentrations is recommended. This study determined that GL has a protective role against motility losses caused by freezing and thawing.

References

1. Bailey JL, Bilodeau J, Cormier N. Semen cryopreservation in domestic animals: A damaging and capacitating phenomenon. J Androl 2000; 21: 1-7.
2. Omur A, Çoyan K. Protective effects of the antioxidants curcumin, ellagic acid and methionine on motility, mitochondrial transmembrane potential, plasma membrane and acrosome integrity in freeze-thawed Merino ram sperm. Vet Med 2016; 1: 10-16.
3. Özer Kaya Ş, Gür S, Kaya E. Effect of l-arginine addition on long-term storability of ram semen. Andrologia 2018; 50: e12945.
4. Türk G, Koca RH, Güngör İH, et al. Effect of hydrated C60 fullerene on lipid, vitamin and amino acid composition in frozen-thawed ram semen. Anim Reprod Sci 2022; 238: 106939.
5. Schiller J, Arnhold J, Glander HJ, Arnold K. Lipid analysis of human spermatozoa and seminal plasma by MALDI-TOF mass spectrometry and NMR spectroscopy-effects of freezing and thawing. Chem Phys Lipids 2000; 106: 145-156.
6. Perez-Pe R, Grasa P, Fernandez-Juan M, et al. Seminal plasma proteins reduce protein tyrosine phosphorylation

in the plasma membrane of cold-shocked ram spermatozoa. Mol Reprod Dev 2002; 61: 226-233.

7. Türk G. Reaktif oksijen türlerinin spermatozoon fonksiyonları üzerindeki fizyolojik ve patolojik etkileri. Turkiye Klinikleri J Reprod Artif Insemin-Special Topics 2015; 1: 26-34.

8. Çoyan K, Başpınar N, Bucak MN, Akalın PP. Effects of cysteine and ergothioneine on post-thawed Merino ram sperm and biochemical parameters. Cryobiology 2011; 63: 1-6.

9. Demir K, Bakırer Öztürk G, Cirit Ü, et al. Effects of cooling rate on membrane integrity and motility parameters of cryopreserved ram spermatozoa. Kafkas Univ Vet Fak Derg 2015; 21: 61-67.

10. Thu ZM, Myo KK, Aung HT, et al. Bioactive phytochemical constituents of wild edible mushrooms from Southeast Asia. Molecules 2020; 25: 1972.

11. Jiang G, Lei A, Chen Y, et al. The protective effects of the Ganoderma atrum polysaccharide against acrylamide-induced inflammation and oxidative damage in rats. Food Funct 2021; 12: 397-407.

12. Oluwafemi Adetuyi B, Olamide Okeowo T, Adefunke Adetuyi O, et al. Ganoderma lucidum from red mushroom attenuates formaldehyde-induced liver damage in experimental male rat model. Biology 2020; 9: 313.

13. Doğan G, İpek H. The protective effect of Ganoderma lucidum on testicular torsion/detorsion-induced ischemia-reperfusion (I/R) injury. Acta Cir Bras 2020; 35: e202000103.

14. Ghajari G, Nabiuni M, Amini E. The association between testicular toxicity induced by Li2Co3 and protective effect of Ganoderma lucidum: Alteration of Bax and c-Kit genes expression. Tissue Cell 2021; 72: 101552.

15. Acısu T.C, Akarsu S.A, Sönmez M, et al. Ameliorative effects of ganoderma lucidum (Reishi) on testicular tissue of rats exposed to bisphenol A. Polish Journal of Veterinary Sciences 2024; 27: 459-467.

16. Placer ZA, Cushman L, Johnson BC. Estimation of products of lipid peroxidation in biological fluids. Anal Biochem 1966; 16: 359-364.

17. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7: 88-95.

18. Aebi H. Catalase. In: Bergmeyer HU (Editor). Methods of Enzymatic Analysis. 2nd Edition, Weinheim: Verlag Chemie, 1974: 673-678.

19. Beutler E. Red Cell Metabolism. A Manual of Biochemical Methods, 3rd. Edition. Orlando: Grune & Stratton; 1984: 310-311.

20. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 130-139.

21. Sun Y, Oberly LW, Ying LA. Simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34: 497-500.

22. Büyük B, Demirci T, Demir N, Türkön H. Can Ganoderma lucidum be an alternative nutritional supplement for enhancing sperm motility rate? Izmir Democ Univ Health Sci J 2021; 4: 47-61.

23. Tuncer SÇ, Akarsu SA, Küçükler S, Gür C, Kandemir FM. Effects of sinapic acid on lead acetate-induced oxidative stress, apoptosis and inflammation in testicular tissue. Environ Toxicol 2023; 38: 2656-2667.

24. Li Y, Liang W, Han Y, et al. Triterpenoids and polysaccharides from Ganoderma lucidum improve the histomorphology and function of testes in middle-aged male mice by alleviating oxidative stress and cellular apoptosis. Nutrients 2022; 14: 4733.

25. Jiang G, Lei A, Chen Y, et al. The protective effects of the Ganoderma atrum polysaccharide against acrylamide-induced inflammation and oxidative damage in rats. Food Funct 2021; 12: 397-407.

26. Yi X, Qiu Y, Tang X, et al. Effect of five different antioxidants on the effectiveness of goat semen cryopreservation. Reprod Sci 2024; 31: 859.

27. Ozmen MF, Seker U, Yavas MC, Cirit U, Akdag MZ. Effects of ganoderma lucidum and melatonin on sperm in rats exposed to electromagnetic field. International Journal of Radiation Research 2021; 19: 121-126.

28. Ghajari G, Chakraborty A, Moosavi SA, Saremi M. The protective effect of ganoderma lucidum in mice-exposed to sertraline. Medicine Personolized Journal Summer 2022; 26: 6-12.