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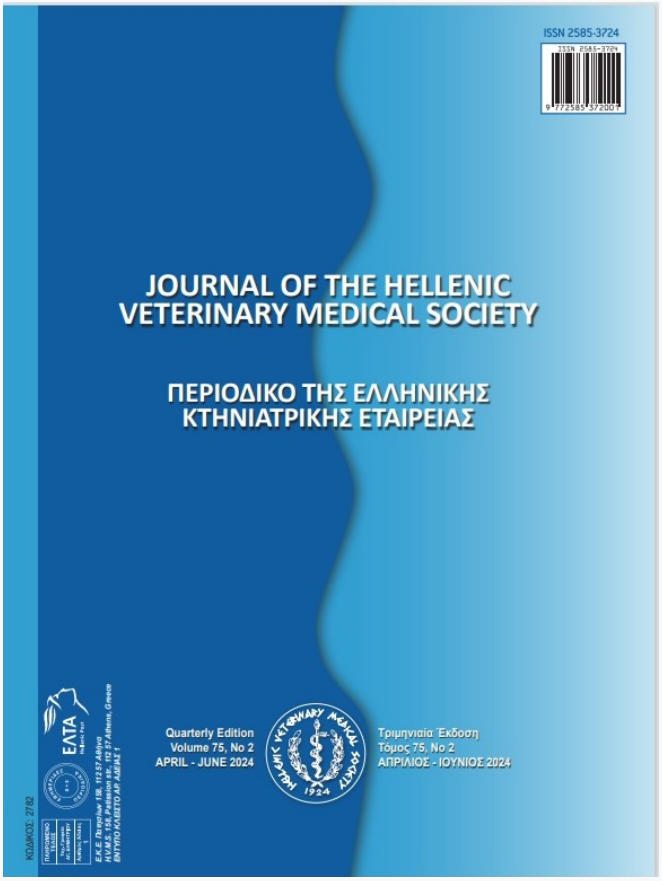

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Research article
Ερευνητικό άρθρο

Effect of ß-glucan from *Euglena gracilis* as an antioxidant on goat semen cryopreservation

B. Ustuner[1*], M.M. Yılmaz[1], O. Huraydin[1], T. Cimen[1], F. Ak Sonat[2], A. Aktar[1], E. Gokce[3], D. Karakci[4], E. Sengul[5], M.K. Soylu[1], Z. Nur[1]

[1]*Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Bursa Uludag University, 16059 Görükle, Bursa, Turkey*

[2]*Department of Physiology, Faculty of Veterinary Medicine, Bursa Uludag University, 16059 Görükle, Bursa, Turkey*

[3]*Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Namık Kemal University, 59030, Tekirdag, Turkey*

[4]*Department of Biochemistry, Faculty of Veterinary Medicine, Namık Kemal University, 59030, Tekirdag, Turkey*

[5]*Department of Breeding Techniques, Sheep Breeding Research Institute, 10200 Bandırma, Balıkesir, Turkey*

ABSTRACT: This study aimed to determine the influence of different doses of β-glucan on post-thaw spermatological parameters, lipid peroxidation, and total antioxidant activity of buck semen. In the non-breeding season, semen was collected from bucks twice weekly. After then, ejaculates were pooled and divided into four equal aliquots: β-glucan concentrations of 1 mM (βG1), 2 mM (βG2), and 4 mM (βG4), and a control group without antioxidants. Each sample group was diluted for cryopreservation using a dilution method involving two steps. The experimental groups were then evaluated for several parameters including sperm motility, plasma membrane functional integrity [hypoosmotic swelling test (HOST)], damaged acrosome rate [FITC-Pisum sativum agglutinin (FITC-PSA)], DNA integrity [terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)], mitochondrial membrane potential using JC-1, evaluation of lipid peroxidation (Malondialdehyde; MDA) and determination of total antioxidant capacity; TAC). The post-thaw motility and plasma functional integrity of the control group were significantly lower than those values in the βG groups ($P < 0.05$). Although the numerically greatest acrosome damage was detected in the control group, it was only statistically different from βG1 and βG4 ($P<0.05$). While the DNA fragmentation rate of the control group was higher than βG4 group ($P<0.05$), it was similar to βG1 and βG2 groups ($P>0.05$). There was no statistical difference among all the groups regarding low mitochondrial membrane potential, MDA, and TAC rates. In line with our results, supplementation of 1mM, 2 mM and 4 mM β-glucan to freezing extender improves the post-thaw spermatological characteristics of goat semen.

Keywords: Goat; ß-glucan; mitochondrial membrane potential; DNA fragmentation; antioxidant activity

Corresponding Author:
Burcu Ustuner, Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Bursa Uludag University, 16059 Görükle, Bursa, Turkey
E-mail address: bbaspinar@uludag.edu.tr

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INTRODUCTION

Successful animal breeding relies on the use of frozen semen from high breeding value bucks for artificial insemination (AI). AI, an assisted reproductive technique, is an important achievement of modern animal husbandry technology as it accelerates breeding studies by preserving genetic resources for many years (Ren et al., 2018).

Some factors contribute to the success of cryopreservation, such as the cryoprotectant or extender used, the cooling rate, and the composition of the extender (Cotter et al., 2005). During cryopreservation, lipid peroxidation (LPO) occurs, and the oxidation of unsaturated fatty acids (UFA) reveals reactive oxygen species (ROS), which causes oxidative damage (Collodel et al., 2022). Sperm cells naturally produce ROS as part of their function, but when production exceeds the cells' ability to defend against it through antioxidant activity, a state of oxidative stress is induced. This oxidative stress can damage the plasma and mitochondrial membranes of spermatozoa. Mitochondrial membrane damage has been shown to cause DNA fragmentation, resulting in reduced sperm fertilization capacity (Aitken and Baker, 2004). To ensure optimal results, it may be necessary to restore normal levels of antioxidants in semen by adding them to extenders during the cryopreservation process. Thus reducing the harmful effects of ROS, potentially leads to an enhancement in the quality of post-thaw semen (Memon et al., 2011).

The β-glucans, as non-starch polysaccharides, have a diverse structure. They are responsible for forming the structural elements of cell walls in various microorganisms, including yeast, algae, certain protists, and also in certain grains like oats and wheat, as well as mushrooms (Barsanti et al., 2011). Previous research shown that β-glucan have a variety of biological activities, such as antioxidation and immunomodulation effects (Dietrich-Muszalska et al., 2011). It is known that β-glucan can prevent oxidative damage to DNA through a scavenging process (Harada and Ohno, 2008; Bashir and Choi, 2017).

Studies demonstrated that ß-glucans could provide advantages in treating different illnesses and conditions such as spinal cord injury (Kayali et al., 2005), sepsis (Sener et al., 2005), brain injury after menopause (Selli et al., 2016), periodontal diseases (Breivik et al., 2005), respiratory problems (Richter et al., 2015), and lung injury (Gulmen et al., 2010) caused by ischemia/reperfusion.

It is known that the administration of β-glucan effectively prevented increases in tissue malondialdehyde (MDA) levels and the decrease in tissue glutathione (GSH) reserves (Sener et al, 2006). Similarly, as noted by several other investigators, it has been demonstrated that β-glucans exert a protective effect against oxidative stress by suppressing the production of cytokines and the activation of pro-inflammatory agents that trigger lipid peroxidation and tissue injury (Kaya et al., 2019).

It was found that all of the tested ß-glucan samples have the ability to form gels. Also, it was indicated that the gelation rate of the ß-glucan samples varied depending on their molecular weight (Mw) (Skendi et al., 2003). Specifically, ß-glucans with lower Mw demonstrated a higher gelation rate than those with higher Mw (Cui and Wood, 2000). The low molecular weight (486.42 g/mol) ß-glucan isolated from *Euglena gracilis* used in the present study was chosen to take advantage of its gel-forming ability. It has been proposed that egg yolk- or soybean lecithin-derived phospholipids may combine with the sperm membrane to produce a safeguarding film that prevents the formation of ice crystals within the cell and protect the sperm membrane from the physical damage during the freeze-thawing process (Quinn et al., 1980).

Ak Sonat et al. (2018) studied the co-administration of ß-glucan with dietary restriction in rats. They have found that ß-glucan is highly effective in improving spermatological parameters and reproductive performance.

But so far, no studies have investigated the antioxidant effect of β-glucan against cryodamage in semen cryopreservation protocols. The objective of this study was to investigate the effect of β-glucan at different doses as an antioxidant supplement in the semen extender on post-thaw spermatological parameters (motility, plasma membrane functional and acrosome integrity, DNA fragmentation, and mitochondrial membrane potential), lipid peroxidation and total antioxidant activity of buck semen. In the present study, besides the β-glucan antioxidative property, its gel-forming ability to defend the sperm membrane during cryopreservation was investigated.

MATERIAL AND METHODS

Ethical approval

All the experimental procedures and evaluation methods used in the study were approved by the Sci-

entific Ethical Committee (Uludag University, Bursa, Turkey; no.2022-03/10) at Uludag University in Bursa, Turkey.

Semen extender and preparation

Tris-based solutions were formulated by adding 1% soybean lecithin (L) (l-α-Phosphatidyl choline from Soybean Type II-S Sigma, P5638). Extender A consisted of 223.7 mmol/L Tris (Sigma, St. Louis, MO, USA), 55.5 mmol/L fructose (Sigma), 66.6 mmol/L citric acid (Merck, Darmstadt, Germany), 4 g/L penicillin G, 3g/L dihydrostreptomycin in distilled water. Extenders B were prepared by adding 100.4 mmol/L trehalose, 4.03 mmol/L EDTA and 6% glycerol (v/v) to extender A (Aisen et al., 2000). To designed for four experimental groups, β-glucan (βG; Sigma 89862, CAS:9051-97) was added at different concentrations (0 mMol, 1 mMol, 2 mMol, or 4 mMol) to the lecithin-based extender A and B solution. The were named as follows: Control (no βG), βG1 (1 mMol βG), βG2 (2 mMol βG), and βG4 (4 mMol βG).

Semen collection, evaluation, dilution, freezing, and thawing

Five Saanen male goats, aged between 3 and 4 years and known to have a history of fertility, were provided with unlimited fresh drinking water and allowed to freely consume maize and alfalfa hay. They were accommodated and maintained by the Veterinary Medicine Faculty at Uludag University, located in Northwestern Anatolia, Turkey.

Twice weekly during the non-breeding season, the semen of the male goats was collected using an electro-ejaculator (Minitube, Germany) and this process was repeated five times. After the collection of the semen, their evaluations were carried out as follows immediately: Once semen was collected, it was put in at a temperature of 30°C water bath, and assessed for its rapid wave motion (using a 0-5 scale), and the motility (%). If the semen had a rapid wave motion (between 3-5 scale), and over 70% initial motility, it was pooled.

The dilution process was conducted by a two-step dilution method. Finally, each 0.25 mL straw has a set containing $125x10^6$ spermatozoa. To achieve this, the pooled ejaculates were divided into four equal parts, and each part was diluted with extender A (Control, βG1, βG2, or βG4). Afterward, sperm cooled down to 5°C within 1 hour. Subsequently, the cooled semen groups were diluted with extender B (Control, βG1, βG2, or βG4) at a 1:1 (semen+extender A: extender B) ratio. A 2-hour equilibration period at 5°C was provided for the semen samples following the addition of the second extender.

The equilibrated semen was placed in 0.25 mL straws and frozen using a Nicool Plus PC freezing machine (Air Liquide, Marne-la-Vallée Cedex 3, France). The process of freezing the semen included a gradual reduction in temperature from +5°C to -8°C, with a cooling rate of 3°C per minute. This was followed by a rapid cooling using liquid nitrogen vapor from -8°C to -120°C, with a cooling rate of 15°C per minute. The frozen straws were then stored in liquid nitrogen at a temperature of -196°C for a minimum of one month. For the evaluation of the post-thaw spermatological parameters, straws were thawed in a water bath (40°C for 30 seconds).

Semen evaluation

Spermatological parameters were evaluated at fresh and post-thaw stages (Figure 1).

Mass activity and motility

Sperm mass activity and motility were subjectively evaluated using a phase-contrast microscope (Olympus BX51) equipped with a heated mechanical stage, as described previously (Ustuner et al., 2018). Percentage of sperm motility was estimated with warm stage at magnification x 400 according to the standard method. It was performed from three different microscopic fields.

The Hypoosmotic Swelling Test (HOST)

The functional integrity of the sperm membrane was evaluated according to Ustuner et al. (2018). HOST which was based on curled and swollen tails, and this test was performed by incubating 10 µL of semen with 100 µL of a 100 mOsM hypoosmotic solution (9 g fructose + 4.9 g sodium citrate per litre of distilled water) at 37 °C for 60 min. After incubation, two hundred sperm cells were evaluated under magnification ×1000 with phase-contrast microscopy. Sperm with swollen or coiled tails were recorded.

Fluorescein Lectin Staining Assay (FITC conjugated Pisum sativum agglutinin (FITC-PSA)

The method described by Nur et al. (2010) was used to assess the integrity of the acrosome. Briefly, 20 µL of diluted semen was resuspended in 500 µL PBS and centrifuged at 100 g for 10 min; the superna-

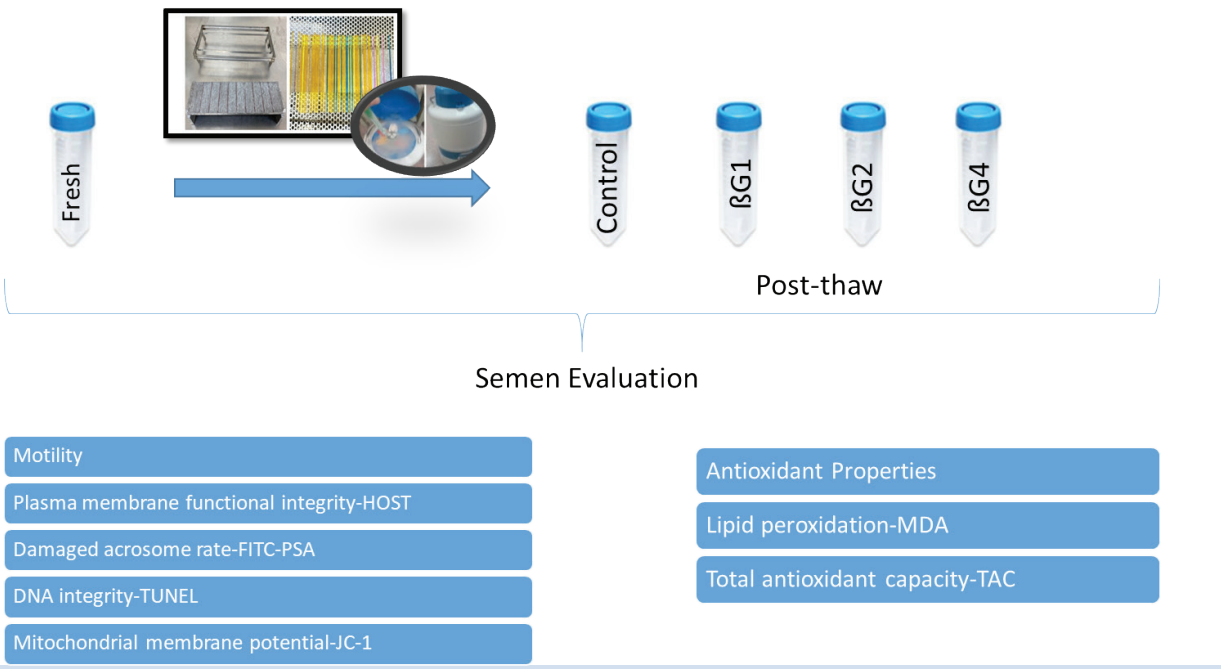

Figure 1. Experimental design. Spermatological parameters (motility, plasma membrane functional and acrosome integrity, DNA integrity, and mitochondrial membrane potential), lipid peroxidation and total antioxidant activity

tant was then discarded. The spermatozoa pellet was resuspended in 250 µL PBS. One drop of resuspended spermatozoa was smeared on a glass microscope slide and air dried. Air-dried slides were fixed with acetone at 4 °C for 10 min, and the slides were covered with PSA-FITC solution (50 mg/mL in PBS solution) and maintained in the dark for 30 min. Stained slides were rinsed with PBS solution, covered with glycerol, and examined under a fluorescence microscope. At least 200 spermatozoa per smear were evaluated for acrosome integrity.

TUNEL (Terminal-deoxynucleotidyl-transferase-mediated-dUTP nick-end labelling) Assay

DNA fragmentation of sperm cells was evaluated using a slightly modified TUNEL technique recommended by the manufacturer (Nur et al., 2010). Following the labeling process, at least 100 spermatozoa were analyzed to determine the percentage of TUNEL (+) spermatozoa, which is an indicator of sperm cells with impaired DNA.

Mitochondrial Membrane Potential (5.5', 6,6'-tetrachloro-1, 1', 3,3'-tetraethyl- benzimidazolo carbocyanine iodide)

To assess the mitochondrial membrane potential in sperm cells, the JC-1 staining method was used previously described by Inanc et al. (2019). A fluorescence microscope (Olympus BX 51-TF) was used to examine at least 100 spermatozoa at 400 magnification. Spermatozoa with yellow-orange dye in the middle were considered to have high mitochondrial membrane potential.

Antioxidant properties

MDA (Malondialdehyde)

Sperm malondialdehyde concentration, as indices of LPO in sperm was measured by microplate reader (Biotek Epoch, USA) using ELISA kit (BT LAB Sheep Malondialdehyde ELISA KIT, Cat.No E0039Sh, China). The sperm samples were prepared following the established procedures and placed in microplate wells coated with MDA antibody. The biotinylated MDA antibody was added to the sample, resulting in binding to the MDA present within the sample. It is then bound to the biotinylated MDA antibody by adding Streptavidin-HRP to the wells. The microplate was incubated for 1 hour at 37°C. Following incubation, any unbound Streptavidin-HRP was eliminated through a washing step. Then, substrate solutions were added, and color change was observed in proportion to the amount of MDA. The reaction was stopped by adding an acidic stop solution and the absorbance was measured at a wavelength of 450 nanometers. The average absorbance was used to construct a standard curve. The calculated results were composed in units of nmol/mL.

TAC (Total antioxidant capacity)

A colorimetric test kit from Elabscience Biotechnology Inc. (Houston, Texas, USA) was utilized according to the manufacturer's guidelines to quantify the overall antioxidant capacity of sperm. The kit contains Fe^{+2}, which is oxidized to Fe^{+3} by the oxidizing compounds present in the sample during the assay. The sperm samples were prepared according to the assay protocol, and then the working solutions and reagents were added and allowed to incubate for 10 minutes at room temperature. The optical density (OD) values of the samples were then determined spectrophotometrically at 520 nm, and the TAC activity was calculated using the formula provided in the kit, in units of U/mL.

Statistical analysis

The study's data was analyzed using SPSS (version 26 for Windows, produced by SPSS in Chicago, IL). To analyze data that satisfied normality and homogeneity assumptions, one-way ANOVA was performed, and group means were compared using Tukey's post hoc test. The Kruskal-Wallis and Mann-Whitney U tests were used for pairwise comparisons of nonparametric sperm parameters.

RESULTS

The freeze-thaw process had a negative impact on several parameters of the pooled fresh semen samples including motility, plasma membrane functional integrity (HOST-positive), acrosome defect (FITC-PSA-positive), DNA integrity (TUNEL-positive), low mitochondrial membrane potential (JC-1-positive) rates, as well as MDA and TAC levels ($P<0.05$).

Table 1 and Figure 2 show the rates of post-thaw goat semen parameters including sperm motility, plasma membrane functional integrity, acrosome defect, DNA fragmentation, and low mitochondrial membrane potential rates of study groups. Meanwhile, Table 2 and Figure 3 present the values of malondialdehyde (MDA) and total antioxidant capacity (TAC) of the four post-thaw groups.

Sperm motility

The post-thaw motility of the control group was notably lower (42.2 ± 5.9%) compared to the βG1 (53.3 ± 7.1%), βG2 (56.1 ± 2.6), and βG4 (54.2 ± 4.4%) groups ($P < 0.05$). There were no statistically significant variations observed in the motility rates among the βG groups, as the results did not reach statistical significance ($P>0.05$).

Plasma membrane integrity

The results of the study showed that the antioxidant groups had significantly higher post-thaw membrane functional integrity compared to the control group ($P<0.05$). No significant difference in sperm membrane functionality was observed with varying doses of the antioxidant ($P > 0.05$).

Defected acrosome rate

The control group had the highest rate of acrosome damage, but it was only significantly different

Table 1. The mean (±SEM) of spermatological parameters in different doses of ß-glucan (1 mMol, 2 mMol, and 4 mMol) and control group.

Groups	Motility (%)	Plasma Membrane Integrity (%)	Defected Acrosome (%)	Fragmented DNA (%)	Low Mitochondrial Memrane Potential (%)
Control	42.25 ± 5.98[a]	59.80 ± 7.73[a]	45.60 ± 3.21[a]	22.09 ± 9.16[a]	21.80 ± 7.19
1mM ß-Glucan	53.33 ± 7.11[b]	72.00 ± 4.06[b]	35.80 ± 2.49[b]	12.89 ± 3.34[ab]	18.40 ± 5.32
2mM ß-Glucan	56.17 ± 2.61[b]	75.30 ± 5.17[b]	38.40 ± 6.27[ab]	13.14 ± 4.31[ab]	16.40 ± 5.81
4mM ß-Glucan	54.25 ± 4.47[b]	74.70 ± 2.39[b]	35.60 ± 4.72[b]	11.43 ± 2.51[b]	15.80 ± 6.06

[a, b] Values with different superscripts in the same column for the effect of different extender groups are significantly different ($p <0.05$). SEM, standard error of mean.

Table 2. The mean (±SEM) antioxidant properties [malondialdehyde concentration (MDA), total antioxidant capacity (TAC)] of goat semen in different doses of ß-glucan (1 mMol,2 mMol, and 4 mMol) and control group.

Groups	MDA (nmol/ml)	TAC (U/ml)
Control	2.85 ± 1.51	14.90 ± 7.79
1mM ß-Glucan	3.78 ± 1.74	11.37 ± 5.53
2mM ß-Glucan	3.99 ± 1.70	12.99 ± 7.26
4mM ß-Glucan	5.67 ± 2.25	12.05 ± 3.12

SEM, standard error of mean.

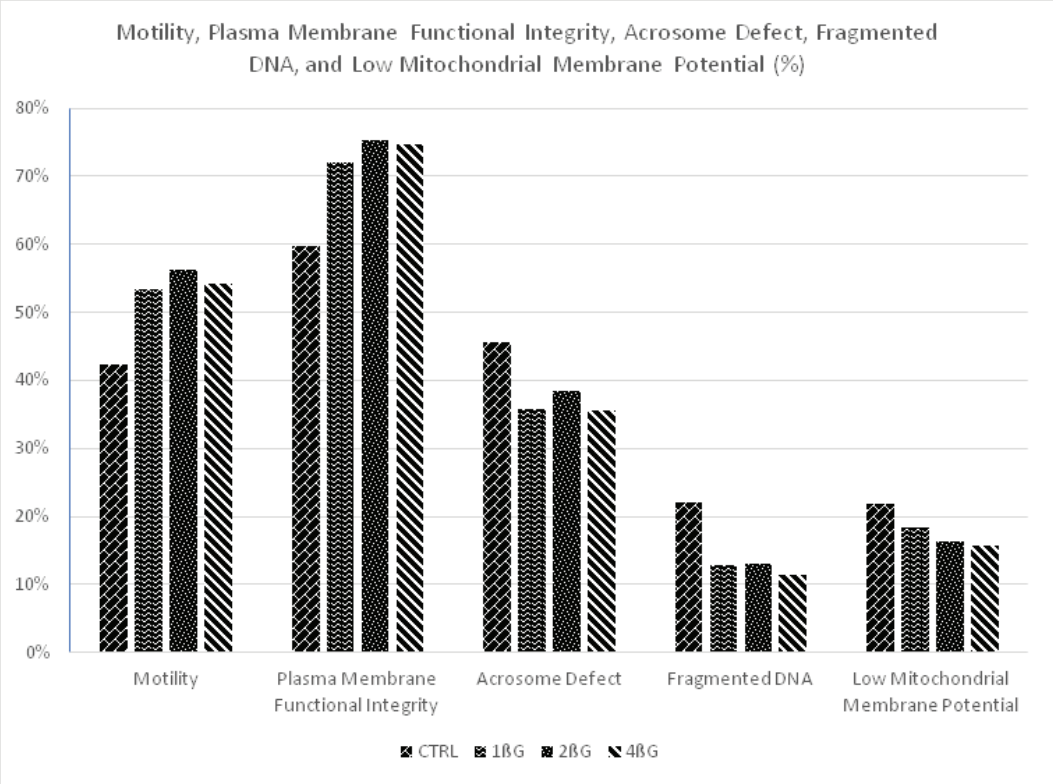

Figure 2. The effect of different doses of ß-glucan on post-thaw goat spermatological parameters. CTRL: Control, (no β-glucan); βG1: 1mMol β-glucan, βG2: 2mMol β-glucan and βG4: 4mMol β-glucan.

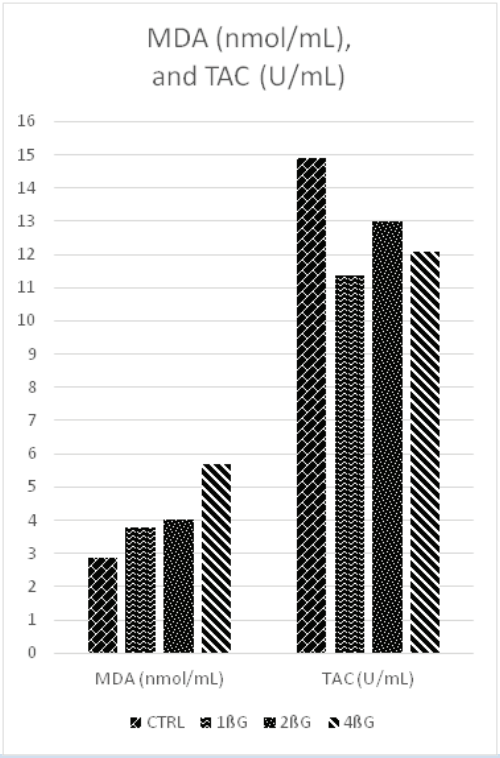

Figure 3. The effect of different doses ß-glucan on post-thaw antioxidant properties [malondialdehyde concentration (MDA), total antioxidant capacity (TAC)] of bucks. CTRL: Control, (no βG); βG1: 1mMol β-glucan, βG2: 2mMol β-glucan and βG4: 4mMol β-glucan.

from the βG1 and βG4 groups (P<0.05) (Figure 4). There were no significant differences in the rates of acrosome defects among the βG groups (P>0.05), as shown in Table 1. and Figure 2.

DNA fragmentation rate

The βG4 group exhibited significantly lower rates of DNA fragmentation compared to the control group (P<0.05), but there were no significant differences observed among the control, βG1 and βG2 groups (P>0.05) (Figure 4). Moreover, no significant differences were found among the βG groups in terms of the percentage of post-thaw sperm DNA fragmentation rates (P>0.05), as depicted in Table 1.

Low mitochondrial membrane potential

There were no significant differences in the rates of low mitochondrial membrane potential among all groups after thawing, although the control group had a slightly higher percentage, as shown in Table 1 and Figure 2.

Malondialdehyde (MDA) concentrations

The impact of the freeze-thaw process on MDA levels in post-thaw semen samples is presented in Ta-

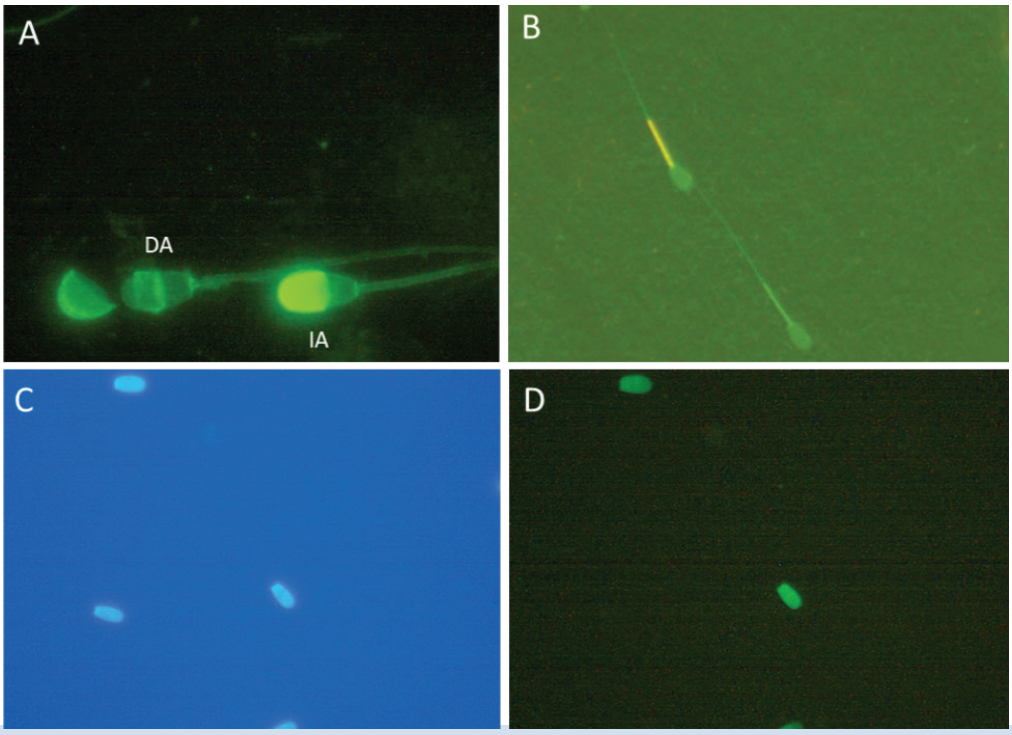

Figure 4. Representative patterns of post-thaw goat sperm as observed with FITC-PSA, JC-1 and TUNEL staining, respectively.

A: FITC-PSA staining for acrosomal membrane integrity evaluation (x100). DA:Defected Acrosome, IA: Intact Acrosome.
B: JC-1 staining for mitochondrial membrane potential assesment. Yellow/orange and green fluorescence associated with midpiece of spermatozoa indicated a high and a low mitochondrial membrane potential, respectively (x40).
C, D: Evaluation of sperm DNA fragmentation using TUNEL assay. C: DNA in both normal and spermatozoa with fragmented DNA in DAPI (blue) and D: DNA fragmented spermatozoa stained green as TUNEL (+) cells, respectively (x40).

ble 2 and Figure 3. The study results indicate that the antioxidant-treated groups had lower levels of MDA compared to the control group. Nonetheless, there was no statistical significance in the difference in MDA levels among all groups.

Total antioxidant capacity (TAC)

According to Table 2, there were no statistically significant differences (P>0.05) in the TAC values among all groups.

DISCUSSION

The cold shock and changes in the osmotic pressure of the environment during cryopreservation create mechanical stress and thus causes the disruption of spermatozoa membrane structure (Noiles et al., 1995). This mechanical stress changes the antioxidant mechanism-ROS balance in favor of ROS (Bucak et al., 2015). Antioxidant substances added to freezing extenders have been reported to have positive effects on post-thaw spermatological characteristics in goats by mitigating the negative effects of oxidative stress induced by reactive oxygen species (ROS) (Memon et al., 2013; Ren et al. 2018; Azimi et al., 2020; Ismail et al., 2020; Merati and Farshad, 2021).

ß-glucan has been previously studied for its antioxidant activities, phagocytosis and cell proliferation as demonstrated in previous research (Lei et al., 2015; Divya et al., 2020). When we look at the literature on andrology in veterinary and human medicine, no study has been found on the effect of ß-glucan on semen freezing. As far as it can be seen, this study represents the initial research of the ß-glucan effects on semen cryopreservation, which could be helpful for the bio-conservation of the Saanen goats and other species.

Cryopreservation leads to oxidative stress, which has deleterious effects on the motility and fertilization potential of spermatozoa and the frequency of DNA damage, resulting in elevated occurrences of single- and double-stranded DNA fragmentation (Alvarez and Storey, 1989; Bell et al., 1993; Aitken et al., 1998). A comparison between the post-thaw results obtained in the current study and fresh pooled semen revealed that the cryopreservation process had an adverse impact (P<0.05) on various parameters, including motility, plasma membrane function, ac-

rosome and DNA integrity, mitochondrial membrane potential, and oxidative stress parameters.

The motility of post-thaw semen may be a reliable indicator of successful cryopreservation and fertilizing capacity of spermatozoa (Mortimer, 2000; Del Olmo et al., 2013). The motility detected in the ß-glucan groups was higher than in the control group. This finding was revealed for the first time in our study ($P<0.05$). This result can be based on two opinions; the first is the antioxidant property. The incorporation of cryoprotective agents in semen extenders has been widely studied to protect sperm cells from cryodamage during the freeze-thaw process. These agents are believed to provide a protective environment for sperm cells by reducing ice crystal formation and preventing osmotic shock-induced cell damage. Moreover, this protective layer may shield the sperm membrane from mechanical damage that may occur during the freeze-thawing process. Our second opinion is that ß-glucan has a gel-like property (Doublier and Wood, 1995), so it has the ability to protect the cell membrane, like lecithin and egg yolk.

Despite the absence of significant differences among βG groups in terms of motility ($P>0.05$), 1 mM ß-glucan did not protect the motility sufficiently compared with the βG2 and βG4 groups. However, motility of 1 mM βG was found to be higher than the post-thaw motility values obtained in research conducted in recent years (Mustofa et al., 2023; Rezaei et al., 2023; Zhang et al., 2023).

During cryopreservation, various factors can alter the lipid composition of cell membranes, leading to loss of integrity and function of the plasma membrane (Holt and Penfold, 2014). This, in turn, can negatively impact sperm motility. Nur et al. (2010) showed a correlation between sperm motility and the functional integrity of the plasma membrane. Our study found that β-glucan groups had higher post-thaw functional sperm plasma membrane integrity compared to the control group, and this was associated with better motility. The dose of antioxidant did not have a significant impact on sperm membrane functionality. Our findings of higher post-thaw functional sperm plasma membrane integrity in β-glucan groups were consistent with the studies of Sun et al. (2020) and Zhang et al. (2022).

Cryopreservation has been reported to induce acrosome reactions or premature capacitation on the report of the literature reviews (Talukdar et al., 2017). According to the study findings, the control group had the highest rate of acrosome damage, but it was only statistically different from the βG1 and βG4 groups ($P<0.05$). No statistical difference was detected among βG groups in terms of acrosome defect ($P>0.05$). Numerous researches about goat semen cryopreservation have reported a range of 21.6% to 52.1% acrosomal defects which is in accordance with the present study (Ustuner et al., 2015; Ustuner et al., 2018; Liang et al., 2023).

The stability of the sperm nucleus is essential for successful reproduction in both humans and animals, as evidenced by numerous studies. When the sperm nucleus is damaged, it can lead to reduced fertilization rates, compromised embryo implantation, and a higher probability of abortion (Aitken et al., 1998; Morris et al., 2002). β-glucans have been reported to exhibit a protective effect against oxidative damage to DNA, possibly by scavenging singlet oxygen or hydroxyl radicals in rat lung cell lines (Slamenová et al., 2003). In the present study, it was determined that the highest DNA fragmentation rate was observed in the control group. It was observed that the addition of β-glucan into the goat semen cryopreservation extender protects sperm DNA integrity, especially in βG4 groups (11.43%) compared to the control group ($P<0.05$). In the presented study, the DNA fragmentation rates of the control and all of the β-glucans groups are consistent with Ustuner et al. (2015) and Longobardi et al. (2020).

The mitochondria play a momentous role in sustaining normal sperm function and energy homeostasis through the process of oxidative phosphorylation and activation of ATP synthase. This is necessary for the acquisition of sperm motility (Ruiz-Pesini et al., 2007; Amaral et al., 2013). Unsaturated fatty acids present in the mitochondrial membrane can lead to disturbance of electron transport chain (ETC) by interrupting the flow of electrons. It is possible that this could bring about an increase in mitochondrial ROS formation, damaging the spermatozoon tail's bioenergy-related pathways (Nowicka-Bauer and Nixon, 2020). Although no difference was found among the groups regarding low mitochondrial membrane potential in the present study, the highest mitochondrial membrane damage observed in the control group was interconnected with a lower motility rate. The same association was detected in the motility of the β-glucan groups, where lower mitochondrial membrane damage was observed.

The antioxidant system has been characterized as a mechanism of defense against lipid peroxidation in semen (Aitken et al., 2012). The present study found that the administration of ß-glucan did not significantly affect MDA and TAC levels, indicating its lack of efficacy in reducing MDA levels and enhancing antioxidant activity. Similarly, Bucak et al. (2009), different antioxidants were added to the extender during cryopreservation of goat semen. The authors reported that certain antioxidant groups resulted in improved post-thaw spermatological parameters, but were also associated with higher MDA levels and lower antioxidant levels. Bucak et al. (2009) reported that the reason for increased MDA and low antioxidant levels caused by differences in the cryopreservation protocol, extender formula, type of antioxidant and concentrations, and animal species-breeds may explain different behaviors of antioxidant capacities. The results obtained from research by Atessahin et al. (2008) and Salmani et al. (2013), on goat semen, supported this opinion.

CONCLUSIONS

In light of the study outcomes, compared to the control group, ß-glucan can improve sperm post-thaw motility, plasma membrane functional integrity, mitochondrial membrane potential, acrosome, and DNA integrity without influencing MDA and total antioxidant capacity. In line with our results, supplementation of 1mM, 2 mM and 4 mM β-glucan to freezing extender improves the post-thaw spermatological characteristics of goat semen. In further studies, analysis such as antioxidant gene expression profile at the molecular level is required to clarify the question marks about the antioxidant mechanism of ß-glucan in sperm cryopreservation.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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