Kocatepe Veterinary Journal

Kocatepe Vet J. (2023) 16(2):166-173

DOI: 10.30607/kvj. 1257228

RESEARCH ARTICLE

Cryopreservation of Ram Semen Using Capsaicin Supplemented Tris Extender

Muhammed Enes İNANÇ[1], Fatih AVDATEK[2], Şükrü GÜNGÖR[1], Mehmet Fuat GÜLHAN[3], Kemal Tuna OLĞAÇ[4], Barış DENK[5], Deniz YENİ[2], Umut TAŞDEMİR[6]*

[1]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Türkiye
[2]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye
[3]Department of Medicinal and Aromatic Plants, Vocational School of Technical Sciences, Aksaray University, Aksaray, Türkiye
[4]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Ankara University, Ankara, Türkiye
[5]Department of Biochemistry, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye
[6]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Türkiye*

ABSTRACT

In this study, it was designed to reveal the effects of capsaicin on oxidative stress and freezability of ram semen. Ejaculates were taken from Sönmez rams and divided into five specimens and diluted with extender at different rates (4 mM, 2 mM, 1 mM, 500 μM) with and without capsaicin (control; C). Semen samples were thawed with a 37°C water bath for 30 seconds for post-thawed analysis. At the end of the study, sperm motility and kinetic parameters, plasma membrane acrosome integrity (PMAI), mitochondrial membrane potential (MMP), DNA damage, oxidant and antioxidant parameters were analyzed. A decrease was observed in the groups containing capsaicin compared to the C in terms of progressive, total motility and kinetic parameters ($p<0.05$). Besides, positive results were not obtained DNA integrity, PMAI and MMP ($p<0.05$). In conclusion; it was determined that capsaicin added to Tris extender did not have a positive effect on oxidative stress and freezing of ram semen.

Keywords: Antioxidant, Capsaicin, Ram, Spermatozoa

***

Kapsaisin Katkılı Tris Sulandırıcı Kullanılarak Koç Spermasının Dondurularak Saklanması

ÖZ

Bu çalışmada, kapsaisinin oksidatif stres ve koç spermasının dondurulabilirliği üzerine etkilerinin ortaya konması tasarlandı. Ejakülatlar Sönmez ırkı koçlardan alınarak beş eşit kısma ayrıldı ve farklı oranlarda (4 mM, 2 mM, 1 mM, 500 μM) kapsaisin içeren ve içermeyen (kontrol) sulandırıcı ile sulandırıldı. Sperma örnekleri çözüm sonu spermatolojik analizler için 37°C sıcaklıklta 30 saniye süre ile çözdürüldü. Çalışma sonunda spermatozoa hareketliliği ve kinetik parametreleri, plazma membran akrozom bütünlüğü (PMAI), mitokondrial membran potansiyeli (MMP), DNA hasarı, oksidan ve antioksidan parametreler analiz edildi. Progresif, total motilite ve kinetik parametreler bakımından kontrol grubuna göre kapsaisin içeren guruplarda azalma görüldü ($p<0.05$). Ayrıca DNA bütünlüğü, PMAI ve MMP'de olumlu sonuçlar elde edilmedi ($p<0.05$). Sonuç olarak; Tris sulandırıcısına ilave edilen kapsaisinin, oksidatif stres ve koç spermasının dondurulması üzerine olumlu bir etki göstermediği belirlendi.

Anahtar Kelimeler: Antioksidan, Kapsaisin, Koç, Spermatozoa

To cite this article: İnanç M.E. Avdatek F. Güngör Ş. Gülhan M.F. Olğaç K.T. Denk B. Yeni D. Taşdemir U. Cryopreservation of Ram Semen Using Capsaicin Supplemented Tris Extender. Kocatepe Vet J. (2023) 16(2):166-173

Submission: 28.02.2023 **Accepted**: 16.05.2023 **Published Online**: 27.05.2023

ORCID ID; *MEİ: 0000-0001-6954-6309, FA: 0000-0003-2345-8826, ŞG: 0000-0003-3460-522X, MFG: 0000-0003-4838-1597, KTO: 0000-0001-9216-7059, BD: 0000-0002-7586-0895, DY: 0000-0002-9105-5677, UT: 0000-0003-2827-1286*

*Corresponding author e-mail: tasdemiru@gmail.com; umuttasdemir@aksaray.edu.tr

INTRODUCTION

Cryopreservation of semen is a method used for the preservation of spermatozoa. This method allows to increase their genetic values and strengthen selected reproductive traits (Salmani et al. 2013). Various methods have been studied for years for the cryopreservation of mammalian spermatozoa. These methods include different freezing methods and using various rates cryoprotectants (Üstüner et al. 2015). Cryopreservation of semen causes vital biological and functional changes in spermatozoa, especially affecting membranes that impair their functional abilities (Chelucci et al. 2015).

Physiological levels of reactive oxygen species (ROS) are considered a signaling force in some cellular physiological processes such as spermatogenesis, capacitation, and acrosome reaction (Sanocka and Kurpisz 2004). Excessive production of ROS such as hydrogen peroxide (H2O2), superoxide anion (O2−), hydroxyl radical (OH) and lipid hydroperoxide (LOOH) are stated to be among the important factors in the pathophysiology of male infertility. Overproduction of these molecules can react with major macromolecules such as proteins, lipids and DNA, creating a pathological condition in cells (Aitken 2017). Spermatozoa protect themselves against oxidative stress thanks to the antioxidants in the seminal plasma (Kim and Parthasarathy 1998). There is a balance between the formation of free radicals and the rate at which they are neutralized by antioxidants, so that the cell is protected from the negative effects of free radicals. If this balance is changed in favor of free radicals, the amount of free radicals increases in the environment. Spermatozoa generally use antioxidant enzyme systems to relieve subtle oxidative stress on their own. However, in cases where the intracellular defense systems are insufficient, cellular macromolecules such as DNA, protein, carbohydrates, and lipids that are sensitive to oxidant damage are damaged (Sabuncuoğlu and Özgüneş 2011). In addition, the freezing process negatively affects spermatological parameters and fertility (Kulaksız and Daşkın 2009). Due to all these factors, researchers focused on adding substances with high antioxidant properties to extenders during semen freezing (Yeni et al. 2022).

Capsaicin (8-methyl-N-vanilyl-6-nonenamide) is an active ingredient responsible for the pungency of hot peppers. It is a potent agonist of transient receptor potential vanilloid 1 (TRPV1) receptors found on nociceptive sensory neurons (Szallasi and Blumberg 1999). It has been reported that capsaicin protects endothelial cells and macrophages against damage caused by low-density lipoprotein Chen et al. (2015), and hepatotoxicity in rats by acting as a direct antioxidant (Manjunatha and Srinivasan 2006). In animal models and clinical studies, capsaicin has been reported to have hypolipidemic effects Joo et al. (2010), as well as antitumor activities that suppress the transcriptional activity of beta-catenin in human colorectal cancer cells Lee et al. (2012) and induce cell death in human breast cancer via mitochondrial pathway and caspase-7 activation (Chang et al. 2011). Besides, it was determined that capsaicin effectively inhibited cell apoptosis in testes in rats with experimental testicular torsion (Sarıoğlu-Buke et al. 2001).

According to the reviewed literature, no study has been found on the freezing of ram semen with capsaicin so far. In this study, it was designed to reveal the effects of capsaicin, which has been determined to have various beneficial effects for animal and human health, on oxidative stress and the freezability of ram semen.

MATERIAL and METHOD

Study Design

This study was approved by the Afyon Kocatepe University Local Ethics Committee on Animal Research, (Approval number and date; 9,533,702/333-December 17, 2020). For the study, four Sönmez rams aged between 2 to 3 were used. The ejaculates in each semen collection from rams are mixed and this process is repeated 9 times. Mixed ejaculates were divided into five aliquots and extended with Tris Based Extender with 15% egg yolk, and 6% glycerol containing four different experimental groups of capsaicin (4 mM, 2 mM, 1 mM, 500 μM) [lot number; MKCC0600-274666, ≥98%]) and control. The highest working solution of capsaicin (4 mM) was dissolved in 1 ml of ethanol (Merck, 99%) and other groups were prepared.

Extended samples were frozen by liquid nitrogen vapor, and stored in a liquid nitrogen container after equilibrated in 4°C for 2 hours. Semen samples were thawed with a 37°C water bath for 30 seconds for post-thawed analysis. Motility, progressive motility, and sperm kinetic parameters were evaluated with computer assissted sperm analyzer (CASA; Sperm Class Analyzer software, SCA® v.4.2; Microptic S.L., Spain) system; Plasma membrane Acrosome Integrity (PMAI) and mitochondrial membrane potential (MMP) were evaluated by flow cytometry (Beckman Coulter, CA, USA) and DNA fragmentation was evaluated by alkaline single-cell gel electrophoresis method (COMET).

Total and Progressive Motility, Kinetic Characteristics

Analysis was conducted by CASA system and a connected phase-contrast microscope (Nikon Eclipse 50i; Japan) with a heating plate, under a negative phase-contrast (Green-filtered, Ph-) with x100 magnification. Velocities of the motile spermatozoa were detected depending on the VCL, as static (<10 μm.s^{-1}), slow (10–45 μm.s^{-1}), medium (45–75 μm.s^{-1}), rapid (>75 μm.s^{-1}), and progressive movements were determined according to spermatozoa which had ≥75% straightness. After thawing, 5 μl of samples were placed on a slide and covered with a cover slide and placed on the heating plate of the microscope at 37°C. For each of the samples, at least 200 spermatozoa were evaluated in six microscopic areas (Olgaç and Akçay 2021). Frozen-thawed semen samples were analyzed in terms of total motility (TMOT, %), progressive motility (PMOT, %),

average path velocity (VAP, μm.s^{-1}), straight-line velocity (VSL, μm.s^{-1}), curvilinear velocity (VCL, μm.s^{1}), linearity (LIN, %), straightness (STR, %), wobble (WOB, %), the amplitude of lateral head displacement (ALH, μm.s^{-1}), beat-cross frequency (BCF, Hz).

Evaluations of Flow Cytometric Analysis

The evaluations were conducted with a CytoFLEX (Beckman Coulter, CA, USA) containing 610±20, 585±42, 525±40 nm emission filters and a 50 mW laser output (488 nm laser beam). An average of 10,000 events were evaluated per each analysis. Spermatoza was selected by determining side scatter area (SSC-A) vs. forward scatter area (FSC-A) with Pseudocolor plot display. Forward Scatter Height (FSC-H) and Forward Scatter Area (FSC-A) were determined to exclude doublets (Bucker et al. 2019).

Stock solutions of stains were prepared with DMSO. 50 μl of aliquots of JC-1 (0.153 mM T3198, molecular probes, Invitrogen) FITC-PNA (100 μg.ml^{-1}, Sigma, L7381), PI [2.99 mM] L7011, (molecular probes, Invitrogen) were stored at -20^{o}C until use.

Plasma Membrane Acrosome Integrity (PMAI)

FITC/PNA-PI staining protocol was used to detect spermatozoon AI (Inanc et al. 2019). The thawed semen samples were diluted in 492 μl of PBS to obtain a spermatozoa concentration of 5x10^{6}. Then, 5 μl of FITC and 3 μl of PI were added to the mixture and incubated at 37^{o}C for 15 min in a dark environment. Apart from non-cellular events (debris), FITC/PNA-PI-the area was named PMAI.

Mitochondrial Membrane Potential (MMP)

The MMP of the sperm was determined using the 5,5',6,6'tetrachloro1,1'3,3'-tetramethyl benzimidazolyl-carbocyanine iodide (JC-1) (Inanc et al. 2019). The thawed sperm sample was diluted to a concentration of 5×10^{6} sperm in 495 μL of PBS. Briefly, 5 μL of JC-1 was added to the mixture and incubated at 37^{o}C for 15 min in a dark environment apart from non-cellular events (debris), spermatozoa were evaluated in terms of high mitochondrial membrane potential (HMMP) status.

Evaluations of DNA fragmentations

To perform Sperm DNA damage analysis, an alkaline single-cell gel electrophoresis method (COMET) was conducted (Gundogan et al. 2010). Stained and committed samples were evaluated via microscope (Olympus CX31) with fluorescence attachment. Spermatozoa were scored using the CometScore software (TriTek, V. 1.5). At least 200 spermatozoa were inspected and analyzed in six different microscopic fields.

Biochemistry

To evaluate lipid peroxidation, we measured the levels of malondialdehyde (MDA) using a method previously reported by (Draper and Hadley 1990). The method involved the reaction of lipid peroxides with thiobarbituric acid and measurement of the absorbance at 532 nm. The calculated amount of MDA was expressed in nmol.ml^{-1}. To measure the activity of glutathione peroxidase (GSH), we used Ellman's method and calculated the amount as mg.dL^{-1} based on the work of (Hissin and Hilf 1976). To assess total antioxidant status (TAS), we used a colorimetric test kit from Relassay Diagnostics, Gaziantep, TR. The kit measured the reduction of the oxidized radical 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) by antioxidant compounds in the samples. The resulting color change was measured at 660 nm in a spectrophotometer, and the TAS was expressed in mmol.L^{-1}. For the measurement of total oxidant status (TOS), we used the same colorimetric test kit from Relassay Diagnostics, Gaziantep, TR. The kit measured the oxidation of reduced Fe $^{+2}$ in the kit to Fe $^{+3}$ by oxidizing compounds, and the results were calculated as μmol.L^{-1} based on spectrophotometric measurements at 660 nm. Finally, we calculated the oxidative stress index (OSI) using the formula OSI = [(TOS) / (TAS x 100)], as described by (Esen et al. 2012).

Statistical Analyses

Prior to conducting significance tests, the normality of the obtained data was assessed using the Shapiro-Wilks test to ensure that parametric test assumptions were met. Levene's test was employed to examine the homogeneity of variances between groups. ANOVA was utilized to control for statistical differences between variables. The Tukey test was then conducted to evaluate differences between the groups. Descriptive statistics, expressed as "mean ± Standard Error Mean" (Mean±SEM), were calculated for each variable and presented. All statistical analyses were performed with a maximum error rate of 5% using the SPSS 13.0 software package. A p-value of less than 0.05 was considered statistically significant.

RESULT

Total Motility and CASA Parameters

The results of CASA motility and kinetic parameters obtained after freezing-thawing were shown in Table 1, although there were statistical differences among the groups, no positive results were obtained in the application groups in terms of progressive and total motility. It was observed that the kinetic parameters VAP, VSL, VCL, BCF, STR, and hyperactivity values were lower in the treatment groups compared to the C ($p<0.05$).

Flow Cytometer Parameters

As shown in Table 2, although results were different among groups, capsaicin treatment caused adverse effects in PMAI and HMMP($p<0.05$).

DNA Damage

As shown in Table 3, it was determined that the addition of capsaicin to the Tris extender did not have a positive effect on DNA damage prevention ($p<0.05$).

Oxidative Stress Parameters

As seen in Table 4, oxidative stress parameters MDA and GSH were not different among the groups ($p>0.05$). Although TAS, TOS, and OSI were different among the groups, the best results were found in the C as in other parameters ($p<0.05$).

Table 1. The mean (±SEM) of motility values in different doses of capsaicin (500 μM, 1mM, 2mM and 4mM) and control group.

Analysis	Control	500 μM	1 mM	2 mM	4 mM	P
Nonprogressive motility (%)	25.26.±2.63[a]	28.90±1.48[a]	30.33±2.24[a]	24.94±2.40[a]	9.82±0.89[b]	*
Progressive motility (%)	30.77±1.71[a]	20.16±1.41[b]	14.79±2.26[c]	6.64±1.48[d]	1.72±0.20[e]	*
Total motility (%)	56.04±2.42[a]	49.07±1.49[ab]	45.13±3.90[b]	31.59±3.10[c]	11.54±1.05[d]	*
VAP (μm/s)	55.24±2.60[a]	43.95±1.40[b]	39.52±1.25[bc]	36.89±1.17[c]	25.13±0.78[d]	*
VSL (μm/s)	84.38±3.10[a]	72.75±1.65[b]	67.25±1.84[b]	59.98±1.58[c]	43.65±1.19[d]	*
VCL (μm/s)	24.45±0.86[a]	20.04±0.92[b]	16.80±1.41[c]	10.71±0.88[d]	1.38±0.18[e]	*
ALH (μm/s)	8.38±0.44[a]	7.94±0.22[a]	7.68 ±0.29[a]	8.42±0.32[a]	6.39±0.21[b]	*
BCF (Hz)	62.72±0.71[a]	59.11±1.07[bc]	57.48±0.66[c]	60.39±0.61[ab]	56.62±1.02[c]	*
LIN (%)	64.93±1.01[ab]	63.06±1.21[abc]	61.62±1.28[bc]	65.96±1.15[a]	60.19±1.92[c]	*
STR (%)	39.55±2.21[a]	30.62±1.34[b]	26.93±1.11[b]	27.09±1.23[b]	17.59±0.70[c]	*
WOB μm s^{-1}	43.36±1.16[a]	39.92±1.49[ab]	37.85±1.15[b]	42.75±1.05[a]	38.06±1.54[b]	*
Hyperactivity μm s-1	3.16±0.03[a]	2.98±0.05[b]	2.84±0.05[b]	2.44±0.02[c]	2.03±0.06[d]	*

[a ,b, c, d, e] Different superscripts within the same row demonstrate significant differences (*p<0.05)

Table 2. The mean (±SEM) of Plasma membrane Acrosome Integrity (PMAI) and high mitochondrial membrane potential (HMMP) rates in different doses of capsaicin (500 μM, 1mM, 2mM and 4mM) and control group.

Analysis	Control	500 μM	1 mM	2 mM	4 mM	P
PMAI	21.70±1.46[a]	18.41±0.08[b]	16.99±0.58[b]	13.23±0.56[c]	11.74±1.54[c]	*
HMMP	15.63±3.10[a]	10.26±0.15[bc]	10.56±0.30[b]	12.65±0.22[ab]	5.83±0.56[c]	*

[a ,b, c] Different superscripts within the same row demonstrate significant differences (*p<0.05)

Table 3. The mean (±SEM) DNA damage values in different doses of capsaicin (500 μM, 1mM, 2mM and 4mM) and control group.

Analysis	Control	500 μM	1 mM	2 mM	4 mM	P
Tail lenght (μm/s)	27.50±0.55^{c}	26.83±0.75^{c}	32.59±0.79^{b}	26.49±1.40^{c}	39.07±0.82^{a}	*
Tail DNA (%)	61.44±0.67^{ab}	57.50±1.31^{b}	61.64±2.54^{ab}	52.89±1.10^{c}	65.70±0.58^{a}	*
Tail moment (μm/s)	17.99±0.67^{d}	20.19±0.77^{c}	23.84±0.54^{b}	21.85±0.53^{c}	26.61±0.80^{a}	*

^{a,b,c} Different superscripts within the same row demonstrate significant differences (*p<0.05)

Table 4. The mean (±SEM) malondialdehyde (MDA), glutathione peroxidase (GSH), total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) in different doses of capsaicin (500 μM, 1mM, 2mM and 4mM) and control group.

Analysis	Control	500 μM	1 mM	2 mM	4 mM	P
MDA (μmol/mL)	5.69±0.06	5.82±0.08	5.80±0.10	5.70±0.05	5.68±0.15	-
GSH (mg/dl)	46.58±2.89	44.27±1.21	43.84±2.30	42.56±0.72	43.33±1.79	-
TAS (mmol/trolox/ml-10^{9} cell/ml)	4.04±0.17^{a}	3.67±0.09^{ab}	3.92±0.27^{a}	3.41±0.12^{bc}	3.02±0.07^{c}	*
TOS (μmol H2O2 Eqv/L)	5.30±0.46^{b}	6.02±024^{ab}	5.94±011^{ab}	6.25±0.18^{a}	6.45±0.23^{a}	*
OSI (AU)	13.15±1.14^{c}	16.46±0.77^{b}	15.43±0.96^{bc}	18.44±0.80^{b}	21.43±1.17^{a}	*

^{a,b,c} Different superscripts within the same row demonstrate significant differences (*p<0.05)
-, No significant difference(p>0.05)

DISCUSSION

The addition of antioxidants to the cryopreservation medium of semen is done to protect spermatozoa against damage to motility, vitality, energy production, and DNA integrity by ROS (Armstrong et al. 1999; Krzyzosiak et al. 2000; Bilodeau et al. 2002). In recent years, different researchers have shown that TRPV1 plays a role in the maturation and function of spermatozoa. TRPV1 has been reported to be found in mature spermatozoa in many species such as duck Majhi et al. (2020), fish Majhi et al. (2013), bull Gervasi et al. (2011), wild boar Maccarrone et al. (2005) and humans (De Toni et al. 2016; Francavilla et al. 2009). It has been observed that this is a protein that induces Ca2+ activation flux and is involved in many biological events, including spermatozoa motility (Kumar et al. 2016). Capsaicin has proven to be a well-known specific agonist of TRPV1 and the sensitivity of TRPV1 to capsaicin is species-specific (Lee et al. 2011; Szolcsányi 2004). In this study, as in total motility, the best results in progressive motility values were obtained in group C ($p<0.05$). In which contradictory results were presented, Hosseini et al. (2020), reported that they administered capsaicin at a dose of 2.5 mg/kg by oral gavage three times a week for two months in their study in which they created experimental varicocele, and they found that the increase in motility value was statistically significant. Chen et al. (2020), indicated positive effects on zebrafish sperm motility and fertility modulated by TRPV1. It was considered that the difference between our study and these studies may be due to the different animal species, route of administration, dose, and form. The findings in our study were in accordance with prior opinion, Park et al. (2017), stated that they conducted a preliminary study to select the most effective and safe capsaicin dose. As a result of this study, they chose 0.33 mg.kg-1 capsaicin, higher doses have toxic effects on spermatogenesis and the capsaicin dose should be carefully implemented for biological therapy in mammals. Obtained results are compatible the study conducted Majhi et al. (2013), informed that administration of capsaicin did not alter motility in fish spermatozoa, and cells mostly did not respond to capsaicin.

AI is extremely important for the integrity of the spermatozoon membrane, cell integrity, and its role in successful fertilization. MMP is evaluated as a parameter related to ATP production and capacitation by spermatozoa mitochondria via oxidative phosphorylation. For this reason, MMP determination is important together with viability assessment (Korkmaz and Çil 2020). Capsaicin provoked detrimental effects in PMAI and MMP ($p<0.05$). In a study in which contradictory results were presented, Claudia et al. (2014), revealed that exposure of spermatozoa cells to capsaicin (at 100 nM and 500 nM doses) during the capacitation period did not have a direct effect on the acrosome reaction. However, it has been suggested that capsaicin activates receptor-specific TRPV1, causing sperm membrane depolarization due to Na+ influx and the consequent opening of voltage-gated calcium channels, consequently, TRPV1 channels modulate the major pathways involved in capacitation (Claudia et al. 2014). In another study, Salahshouri et al. (2022), claimed that capsaicin has positive effects on fertility and has therapeutic potential by increasing the decreased TRPV1, II, and III. level varicocele diagnosed men.

As with other parameters examined, capsaicin did not show a positive effect on DNA integrity ($p<0.05$). In a study in which compatible results were here Hosseini et al. (2020), speculated that there was no difference in terms of DNA damage in the group given capsaicin in rats with experimental varicocele. Unlike the current study, in the freezing of ram semen by our study group using various antioxidants, it was shown that hesperidin and thymoquinone can protect spermatozoa from DNA damage, especially in freezing processes (Yeni et al. 2022; İnanç et al. 2022). Previous studies with positive results on DNA damage have shown that antioxidants play an important role in gene expression regulated by the sperm epigenome through regulation of DNA methylation (Choucair et al. 2018). Unlike the results of these studies, it was interpreted that capsaicin did not have a positive effect on DNA damage, due to its failure to show its antioxidant properties at the low temperature that occurs during the sperm freezing process.

In our study, although there was no statistical change among the groups in the secondary redox parameters MDA and GSH values ($p>0.05$). However, it was observed that the total redox parameters TAS and TOS showed a balance in favor of oxidants in all groups compared to the C, confirming the pro-oxidant activity ($p<0.05$). Gosh et al. (1993), reported that low doses of capsaicin treatment caused a significant increase in ultraviolet (UV) induced lipid peroxidation by showing pro-oxidant properties, while high doses caused a significant decrease in UV-induced peroxidation with its antioxidant properties. Pro-oxidant agents are compounds that can trigger a series of oxidative reactions that lead to the unfolding of proteins and DNA damage as double-strand breaks. This suggests that capsaicin may have acted as a prooxidant at the dose we used in our study.

CONCLUSION

In ram semen freezing studies, many antioxidant substances have been tested. While sometimes positive results were obtained after these treatments, sometimes appropriate results could not be obtained as presented study. For this reason, it is important to elucidate the bioactive components, detailed chemical structures, behavior patterns, animal species differences, and effective doses of the agents used in the studies. In light of the results obtained, our results suggested that

capsaicin added to the semen extender did not show any improvement or positive effect in terms of sperm motility, kinetic parameters, oxidative stress parameters and DNA damage on ram semen freezing.

Conflict of interest: The authors declared that there are no actual, potential, or perceived conflicts of interest for this article.

Authors Contribution Rate: Data curation, ¸Ş.G.; Methodology, U.T.; Investigation, D.Y., ¸Ş.G., F.A., M.F.G., K.T.O., M.E.İ., B.D. and U.T.; Resources, U.T., D.Y., ¸Ş.G., F.A., M.F.G. and M.E.İ.; Writing original draft, U.T. and D.Y.; Writing review and editing, U.T.; Project administration, U.T. All authors have read and agreed to the published version of the manuscript.

Ethics Committee Information: Afyon Kocatepe University Faculty of Veterinary Medicine Animal Care Committee with the decision numbered 49533702/333.

Financial support: This study was supported by Aksaray University Scientific Research Fund (BAP2021/008). The funders had no role in this study's design, data collection, or analyses.

Acknowledgement: The authors would like to thank Kapucuoğlu sheep farm for the use of their facilities and herd.

REFERENCES

Aitken, R. J. (2017). Reactive oxygen species as mediators of sperm capacitation and pathological damage. Molecular reproduction and development, 84(10), 1039-1052.

Armstrong, J. S., Rajasekaran, M., Chamulitrat, W., Gatti, P., Hellstrom, W. J., & Sikka, S. C. (1999). Characterization of reactive oxygen species induced effects on human spermatozoa movement and energy metabolism. Free Radical Biology and Medicine, 26(7-8), 869-880.

Bilodeau, J. F., Blanchette, S., Cormier, N., & Sirard, M. A. (2002). Reactive oxygen species-mediated loss of bovine sperm motility in egg yolk Tris extender: protection by pyruvate, metal chelators and bovine liver or oviductal fluid catalase. Theriogenology, 57(3), 1105-1122.

Bucher, K., Malama, E., Siuda, M., Janett, F., & Bollwein, H. (2019). Multicolor flow cytometric analysis of cryopreserved bovine sperm: a tool for the evaluation of bull fertility. Journal of dairy science, 102(12), 11652-11669.

Chang, H. C., Chen, S. T., Chien, S. Y., Kuo, S. J., Tsai, H. T., & Chen, D. R. (2011). Capsaicin may induce breast cancer cell death through apoptosis-inducing factor involving mitochondrial dysfunction. Human & experimental toxicology, 30(10), 1657-1665.

Chelucci, S., Pasciu, V., Succu, S., Addis, D., Leoni, G. G., Manca, M. E., & Berlinguer, F. (2015). Soybean lecithin–based extender preserves spermatozoa membrane integrity and fertilizing potential during goat semen cryopreservation. Theriogenology, 83(6), 1064-1074.

Chen, K. S., Chen, P. N., Hsieh, Y. S., Lin, C. Y., Lee, Y. H., & Chu, S. C. (2015). Capsaicin protects endothelial cells and macrophage against oxidized low-density lipoprotein-induced injury by direct antioxidant action. Chemico-Biological Interactions, 228, 35-45.

Chen, Y., Wang, H., Wang, F., Chen, C., Zhang, P., Song, D., & Zeng, X. (2020). Sperm motility modulated by Trpv1 regulates zebrafish fertilization. Theriogenology, 151, 41-51.

Choucair, F., Saliba, E., Abou Jaoude, I., & Hazzouri, M. (2018). Antioxidants modulation of sperm genome and epigenome damage: Fact or fad? Converging evidence from animal and human studies. Middle East Fertility Society Journal, 23(2), 85-90.

Claudia, C., Horatiu, S., Iudith, I., & Constanta, B.V.S. (2014). Research regarding the role of TRPV1 and capsaicin (CPS) implication for capacitation and acrosome reaction. Romanian Biotechnological Letters, 19(3), 9437.

De Toni, L., Garolla, A., Menegazzo, M., Magagna, S., Di Nisio, A., Šabović, I., & Foresta, C. (2016). Heat sensing receptor TRPV1 is a mediator of thermotaxis in human spermatozoa. PloS one, 11(12), e0167622.

De, A. K., Ghosh, J. J., & Mandal, T. K. (1993). Ultraviolet radiation-induced lipid peroxidation in liposomal membrane: Modification by capsaicin. Phytotherapy Research, 7(1), 87-89.

Draper, H. H., & Hadley, M. (1990). Malondialdehyde determination as index of lipid Peroxidation. In Methods in enzymology. Academic press, 186, 421-431.

Esen, Ç., Alkan, B. A., Kırnap, M., Akgül, Ö., Işıkoğlu, S., & Erel, Ö. (2012). The effects of chronic periodontitis and rheumatoid arthritis on serum and gingival crevicular fluid total antioxidant/oxidant status and oxidative stress index. Journal of periodontology, 83(6), 773-779.

Francavilla, F., Battista, N., Barbonetti, A., Vassallo, M. R. C., Rapino, C., Antonangelo, C., & Maccarrone, M. (2009). Characterization of the endocannabinoid system in human spermatozoa and involvement of transient receptor potential vanilloid 1 receptor in their fertilizing ability. Endocrinology, 150(10), 4692-4700.

Gervasi, M. G., Osycka-Salut, C., Caballero, J., Vazquez-Levin, M., Pereyra, E., Billi, S., & Perez-Martinez, S. (2011). Anandamide capacitates bull spermatozoa through CB1 and TRPV1 activation. PLoS One, 6(2), e16993.

Gundogan, M., Yeni, D., Avdatek, F., & Fidan, A. F. (2010). Influence of sperm concentration on the motility, morphology, membrane and DNA integrity along with oxidative stress parameters of ram sperm during liquid storage. Animal Reproduction Science, 122(3-4), 200-207.

Hissin, P. J., & Hilf, R. (1976). A fluorometric method for determination of oxidized and reduced glutathione in tissues. Analytical biochemistry, 74(1), 214-226.

Hosseini, M., Tavalaee, M., Rahmani, M., Eskandari, A., Shaygannia, E., Kiani-Esfahani, A., & Nasr-Esfahani, M. H. (2020). Capsaicin improves sperm quality in rats with experimental varicocele. Andrologia, 52(11), e13762.

İnanç, M. E., Güngör, Ş., Öztürk, C., Korkmaz, F., Baştan, İ., & Çil, B. (2019). Cholesterol-loaded cyclodextrin plus trehalose improves quality of frozen-thawed ram sperm. Veterinarni Medicina, 64,(03), 118–124.

İnanç, M. E., Güngör, S, Avdatek, F., Yeni, D., Gülhan, M. F., Olgaç, K. T., Denk, B., & Taşdemir, U. (2022). Thymoquinone improves motility, plasma membrane integrity and DNA integrity of frozen–thawed ram semen. Andrologia, 54(10), e14547.

Joo, J. I., Kim, D. H., Choi, J. W., & Yun, J. W. (2010). Proteomic analysis for antiobesity potential of capsaicin on white adipose tissue in rats fed with a high fat diet. Journal of proteome research, 9(6), 2977-2987.

Kim, J. G., & Parthasarathy, S. (1998, December). Oxidation and the spermatozoa. In Seminars in reproductive endocrinology. Thieme Medical Publishers, Inc., 16,(04),235-339.

Korkmaz, F., & Çil, B. (2020). Akış Sitometrisinin (Flow Cytometry) Sperma Kalite Analizlerinde Kullanımı. Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 15(1), 76-83.

Krzyzosiak, J., Evenson, D., Pitt, C., Jost, L., Molan, P., & Vishwanath, R. (2000). Changes in susceptibility of bovine sperm to in situ DNA denaturation during prolonged incubation at ambient temperature under conditions of exposure to reactive oxygen species and nuclease inhibitor. Reproduction, Fertility and Development, 12(6), 251-261.

Kulaksız, R., & Daşkın A. (2009). Farklı antioksidanlarla dondurulan Saanen teke spermasının in vitro ve in vivo değerlendirilmesi. Ankara Üniv Vet Fak Derg, 56, 201-205.

Kumar, A., Majhi, R. K., Swain, N., Giri, S. C., Kar, S., Samanta, L., & Goswami, C. (2016). TRPV4 is endogenously expressed in vertebrate spermatozoa and regulates intracellular calcium in human sperm. Biochemical and biophysical research communications, 473(4), 781-788.

Lee, S. H., Richardson, R. L., Dashwood, R. H., & Baek, S. J. (2012). Capsaicin represses transcriptional activity of β-catenin in human colorectal cancer cells. The Journal of nutritional biochemistry, 23(6), 646-655.

Lee, Y. M., Kang, S. M., Lee, S. R., Kong, K. H., Lee, J. Y., Kim, E. J., & Chung, J. H. (2011). Inhibitory effects of TRPV1 blocker on UV-induced responses in the hairless mice. Archives of dermatological research, 303, 727-736.

Maccarrone, M., Barboni, B., Paradisi, A., Bernabò, N., Gasperi, V., Pistilli, M. G., & Mattioli, M. (2005). Characterization of the endocannabinoid system in boar spermatozoa and implications for sperm capacitation and acrosome reaction. Journal of Cell Science, 118(19), 4393-4404.

Majhi, R. K., Kumar, A., Giri, S. C., & Goswami, C. (2020). Differential expression and localization of thermosensitive Transient Receptor Potential Vanilloid (TRPV) channels in the mature sperm of white pekin duck (Anas platyrhynchos). bioRxiv, 2020-02.

Majhi, R. K., Kumar, A., Yadav, M., Swain, N., Kumari, S., Saha, A., & Goswami, C. (2013). Thermosensitive ion channel TRPV1 is endogenously expressed in the sperm of a fresh water teleost fish (Labeo rohita) and regulates sperm motility. Channels, 7(6), 483-492.

Manjunatha, H., & Srinivasan, K. (2006). Protective effect of dietary curcumin and capsaicin on induced oxidation of low-density lipoprotein, iron-induced hepatotoxicity and carrageenan-induced inflammation in experimental rats. The FEBS journal, 273(19), 4528-4537.

Olğaç, K. T., & Akçay, E. (2021). Effects of Spermine and Spermidine supplemented extenders on post-thaw Spermatological Parameters in Stallion Semen Cryopreservation. Cryobiology, 100, 72-76.

Park, S. G., Yon, J. M., Lin, C., Gwon, L. W., Lee, J. G., Baek, I. J., & Nam, S. Y. (2017). Capsaicin attenuates spermatogenic cell death induced by scrotal hyperthermia through its antioxidative and anti-apoptotic activities. Andrologia, 49(5), e12656.

Sabuncuoğlu, S., & Özgüneş, H. (2011). Kemoterapi, serbest radikaller ve oksidatif stres. Hacettepe University Journal of the Faculty of Pharmacy, (2), 137-150.

Salahshouri, S., Akbarian, F., Tavalaee, M., Seifati, S. M., & Nasr-Esfahani, M. H. (2022). Expression of TRPV1 as A Heat Sensitive Voltage-Dependent Ion Channel and Oxidative Stress in Sperm Samples of Infertile Men with Varicocele: A Case-Control Study. Cell Journal (Yakhteh), 24(6), 323.

Salmani, H., Nabi, M. M., Vaseghi-Dodaran, H., Rahman, M. B., Mohammadi-Sangcheshmeh, A., Shakeri, M., ... & Zhandi, M. (2013). Effect of glutathione in soybean lecithin-based semen extender on goat semen quality after freeze-thawing. Small ruminant research, 112(1-3), 123-127.

Sanocka, D., & Kurpisz, M. (2004). Reactive oxygen species and sperm cells. Reproductive biology and endocrinology, 2(1), 1-7.

Sarioglu-Buke, A., Erdem, S., Gedikoglu, G., Bingol-Kologlu, M., & Tanyel, F. C. (2001). Capsaicin effectively prevents apoptosis in the contralateral testis after ipsilateral testicular torsion. BJU international, 88(7), 787-789.

Szallasi, A., & Blumberg, P. M. (1999). Vanilloid (Capsaicin) receptors and mechanisms. Pharmacological reviews, 51(2), 159-212.

Szolcsányi, J. (2004). Forty years in capsaicin research for sensory pharmacology and physiology. Neuropeptides, 38(6), 377-384.

Üstüner, B., Nur, Z., Alcay, S., Toker, M. B., Sağırkaya, H., & Soylu, M. K. (2015). Effect of freezing rate on goat sperm morphology and DNA integrity. Turkish Journal of Veterinary & Animal Sciences, 39(1), 110-114.

Yeni, D. Güngör, Ş. Avdatek, F. Gülhan, M.F. Olğaç, K.T. İnanç, M.E. Denk, B. & Taşdemir, U. (2022). Investigation of Changes in Spermatozoon Characteristics, Chromatin Structure, and Antioxidant/Oxidant Parameters after Freeze-Thawing of Hesperidin (Vitamin P) Doses Added to Ram Semen. Life, 12, 1780.