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The effect of ellagic acid on rabbit sperm *in vitro* parameters after cryopreservation

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Abstract: Cryopreservation reduces the antioxidant activity of spermatozoa and makes them more sensitive to damage caused by reactive oxygen species (ROS). The addition of antioxidants to the freezing medium could prevent cryo-damage by mitigating the harmful effects of ROS and, thus, protecting the spermatozoa. This study aimed to evaluate the effect of ellagic acid (EA) on the rabbit sperm traits after freezing-thawing. Semen samples collected from New Zealand White rabbit males were cryopreserved in a BotuCrio freezing medium (Nidacon, Sweden) supplemented with different concentrations of ellagic acid (EA at 0, 0.5, 1.5 and 2.5 mM) using the manual slow freezing procedure. After thawing, sperm motility parameters were evaluated by CASA. The parameters of viability (DRAQ7), apoptosis (Yo-Pro-1), acrosome integrity (peanut agglutinin; PNA), intracellular ROS (Cell-ROX) and mitochondrial activity (MitoTracker) were evaluated by flow cytometry. EA added to the freezing medium at all concentrations led to a significant reduction ($P < 0.05$) in intracellular ROS in frozen-thawed sperm cells. However, this effect was not reflected in motility parameters. Semen supplemented with 1.5 mM EA also yielded a lower proportion of apoptotic cells compared to the control group. In conclusion, EA supplementation of semen extender demonstrated its antioxidative properties protecting spermatozoa against oxidative damage during cryopreservation. Nevertheless, to draw a definitive conclusion regarding the effect of EA on spermatozoa functionality, additional research is necessary.

Keywords: spermatozoa; antioxidant; viability; motility; ROS

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Rabbits represent an important animal species that affects the fulfilment of the needs of the growing world population due to its high reproduction rate, high genetic selection potential, fast growth, early maturation, good feed conversion and high meat quality (Vizzarri et al. 2019). Artificial insemination with rabbit sperm after cryopreservation was used only for gene bank purposes or for experimental purposes, due to the problems with the use of frozen-thawed sperm, such as lower fertility and variability of results (Moce and Vicente 2009).

Various factors during the freezing process including diluents, sperm preparation and freezing techniques, antioxidants, sudden temperature changes, ice formation and osmotic stress, have been proposed as reasons for poor sperm quality after thawing. Although great progress has been achieved in the field of rabbit sperm cryopreservation, new methodological approaches are needed to overcome the problem of lower sperm viability (Kubovicova et al. 2021).

The most common issue during the sperm cooling process is damage to the plasma membrane as a result of lipid peroxidation (LPO), attributable to the high content of polyunsaturated fatty acids in their membrane. Mammalian spermatozoa are rich in unsaturated fatty acids and easily undergo the reactive oxygen species (ROS) formation that can reduce sperm motility and interfere with the acrosome reaction and sperm capacitation (Sikka 1996). Various antioxidant substances can be used to prevent the formation of ROS and avoid the oxidation process. In its composition, sperm contains antioxidants such as taurine, catalase, glutathione (GSH), glutathione peroxidase (GPx) and superoxide dismutase, which can suppress LPO and excessive formation of ROS (Holt 2000). The phenolic compounds have received much attention due to their wide range of biological activities, such as antioxidant activity, ability to scavenge radicals, chemoprevention (Hassoun et al. 1997; Hassoun et al. 2004; Atessahin et al. 2006; Turk et al. 2008; Turk et al. 2010a, b; Ceribasi et al. 2010) and anti-apoptotic (Turk et al. 2010a) properties. It is well known that ellagic acid (EA) not only has cryoprotective and antioxidant properties but also participates in reducing LPO and increasing the level of total GSH and GPx in rats (Hassoun et al. 2006). Moreover, EA significantly ameliorated damage to ram sperm parameters, oxidant/antioxidant balance and testicular apoptosis induced by chemotherapeutic agents such as cisplatin (Turk et al. 2008) and cyclophosphamide (Turk et al. 2010a).

In rams, the addition of EA at 1 mM resulted in higher motility and viability of spermatozoa after 0 h of storage, while at a dose of 2 mM it led to higher motility and vitality and increased antioxidant potential after 24 and 72 h (Bucak et al. 2019). According to Najafi et al. (2019) 1 mM of EA is an optimal concentration that can improve the quality of rooster sperm after thawing.

As far as we know, no research has been conducted to compare the effect of ellagic acid at different doses on rabbit sperm after cryopreservation. Therefore, the aim of this study was to evaluate the effects of ellagic acid at various doses added to diluted sperm to monitor sperm viability, apoptotic changes, acrosome integrity, mitochondrial membrane potential, and reactive oxygen species parameters after the cryopreservation process.

MATERIAL AND METHODS

Animals and semen collection

Sexually mature New Zealand White broiler rabbit males (n = 9) of M91 and P91 lines without overt evidence of genital tract infections were used in experiments. The rabbits were reared at the breeding facility (NPPC, RIAP Nitra, Lužianky, Slovak Republic) and were housed in individual cages, fed a commercial diet (KV; TEKRO Nitra, s.r.o., Nitra, Slovakia) and received water *ad libitum*. The photoperiod was set at 14 h light and 10 h dark. The temperature and humidity in the area were 17–20 °C and 60–65%, respectively. The experiments were carried out in accordance with the Code of Ethics of the EU Directive 2010/63/EU for animal experiments (https://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm).

The semen samples were collected from rabbit males twice a week with a pre-heated artificial vagina during two months. The semen was transported within 10 min to the laboratory in a water bath at 37 °C as described previously (Krockova et al. 2016).

Semen processing

Semen concentration and motility were immediately measured using a CASA system with

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SpermVision™ Software (MiniTube, Tiefenbach, Germany). Only semen samples with acceptable characteristics (> 60% progressive motility; > 1.0×10^9 spermatozoa/ml and < 10% of abnormal sperm forms) were selected for this study, while samples that had a lower quality were excluded.

After initial evaluation, semen samples were pooled. The pooled ejaculates were centrifuged at 480 × *g* for 10 min at room temperature (RT) to obtain a pellet.

Then the sperm samples were cryopreserved using a manual slow freezing procedure. Briefly, the pellet was re-suspended in a BotuCrio freezing medium (Nidacon, Mölndal, Sweden) and the ejaculate was divided into four equal samples assigned to the experimental groups that contained EA at 0 mM (control), 0.5 mM, 1.5 mM and 2.5 mM, with the final sperm concentration of approximately 500×10^6 cells/ml (Kulikova et al. 2017). The samples were subsequently filled into 0.25 ml straws (Minitüb, Tiefenbach, Germany) and immediately placed into a fridge (4–5 °C) and equilibrated for 0.5 h. The equilibrated straws were placed 4 cm above the surface of liquid nitrogen for 15 min (–125 to –130 °C) and subsequently plunged directly into liquid nitrogen and stored for one week until thawing.

Semen quality evaluation

The straws were thawed in a water bath at 37 °C for 30 s and their content was transferred to an Eppendorf tube pre-heated at 37 °C. Frozen-thawed spermatozoa were evaluated for the same traits as for fresh semen, as described below.

Motility analyses

Total and progressive sperm motility was evaluated using a CASA system with SpermVision™ Software. Briefly, each semen sample was analysed for average concentration (10^9 sperm/ml), percentage of totally motile sperm (motility > 5 µm/s) and percentage of progressively motile sperm (motility > 20 µm/s). Samples were diluted with saline (0.9% NaCl; Braun, Nuaille, Germany) at a ratio of 1 : 10 (vol/vol). Ten µl of pre-diluted fresh and frozen-thawed semen samples were transferred to the Makler counting chamber (Sefi Medical Instruments, Haifa, Israel) and analysed using the SpermVision™ Software under AxioScope A1 light microscope (Carl Zeiss Slovakia, Bratislava, Slovakia). Motility parameters were as follows: total motility (%), progressive motility (%), curvilinear velocity (VCL, µm/s), straight-line velocity (VSL, µm/s), average path velocity (VAP, µm/s), amplitude of lateral head displacement (ALH, µm/s), linearity (LIN, %), straightness (STR, %) and wobble (WOB, %). Sperm parameters were analysed in six microscopic view fields.

Flow cytometry analyses

Aliquots of semen samples from each group were stained using specific chemicals to identify various physiological cell attributes. All chemicals were sourced from ThermoFisher Scientific (USA) unless specified otherwise. Fresh and post-thaw semen samples were incubated with the above-mentioned reagents either following the producer's manuals or as described previously (Vasicek et al. 2022).

To assess apoptotic-like alterations in spermatozoa, we used the Yo-Pro-1 nuclear green stain. We prepared the semen samples by diluting them to contain 1×10^6 spermatozoa in a 500 µl Ca- and Mg-free PBS (Biosera, Cholet, France) solution. These were then treated with 0.5 µl of Yo-Pro-1, achieving a final concentration of 100 nM. The samples underwent a 15-minute incubation period in darkness at room temperature. After incubation, the samples were purified using PBS through centrifugation at 480 × *g* for 10 min at RT.

The evaluation of acrosome integrity was conducted using PNA (peanut agglutinin). One µl volume of the prepared PNA working solution, at a concentration of 0.5 mg/ml, was incubated with a diluted semen sample containing 1×10^6 spermatozoa in 200 µl of PBS. This incubation was carried out for 15 minutes in darkness at room temperature. The PNA working solution was formulated by dissolving the protein, initially at a concentration of 1 mg/ml, into 2 ml of deionized water. Following the incubation, the samples were centrifuged (480 × *g* for 10 min at RT) to wash them.

To evaluate mitochondrial activity, we measured the mitochondrial membrane potential (MMP) using MitoTracker® Green FM (MT Green). In this process, a semen sample containing 1×10^6 sper-

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matozoa was diluted in 500 µl of PBS and then incubated with MT Green dye, achieving a final concentration of 200 nM. This incubation was conducted in darkness at 37 °C for a duration of 10 minutes. Following this, the samples underwent a washing process (480 × *g* for 10 min at RT).

The intracellular ROS level was measured using the CellROX Green assay. A semen sample with 1 × 10^6 spermatozoa was diluted in 500 µl of PBS. This diluted sample was then incubated with CellROX to achieve a final concentration of 2.5 µM. The incubation was carried out at 37 °C for 30 minutes.

All the samples that had been specifically stained and washed were subsequently stained using pre-prepared DRAQ7 (BioStatus, Limited, Shepshed, UK), reaching a final concentration of 3 µM, a far-red fluorescent nucleic acid dye, which stains the nuclei of dead or membrane-compromised cells. These samples were then incubated for 10 min in darkness at room temperature. Following this incubation, the stained sample aliquots were immediately analysed by flow cytometry using a FACSCalibur instrument (BD Biosciences, San Jose, CA, USA) equipped with a 488 nm argon ion laser and red diode (635 nm) laser. Fluorescent signals were acquired by Cell Quest Pro™ software (BD Biosciences, San Jose, CA, USA) in green FL1 channel using 530/30 nm band pass filter and red FL3 channel using 670 nm long pass filter. Each sample was analysed for 10 000 events at least (spermatozoa). Gating strategy is presented in Figure 1.

Statistical analyses

The experiments were repeated four times. Results are expressed as mean ± SEM. Mean values were analysed by analysis of variance (ANOVA) followed by the Tukey post-hoc test to determine the significance of all parameters within all groups using the TIBCO Statistica computer program (v14.0.0.15). *P*-values at $P < 0.05$ were considered statistically significant.

RESULTS

Effect of EA on sperm motility

Average values of motility parameters of rabbit spermatozoa after storage in liquid nitrogen were evaluated (Table 1). Cryopreservation of rabbit sperm with EA at different amounts showed no significant differences in the motility parameters compared to the control group. Average total motility after the EA treatment was slightly higher but this effect was not significant.

Effect of EA on spermatozoa traits assessed by flow cytometry

Table 2 presents the outcomes of the analysis encompassing dead spermatozoa (DRAQ7+),

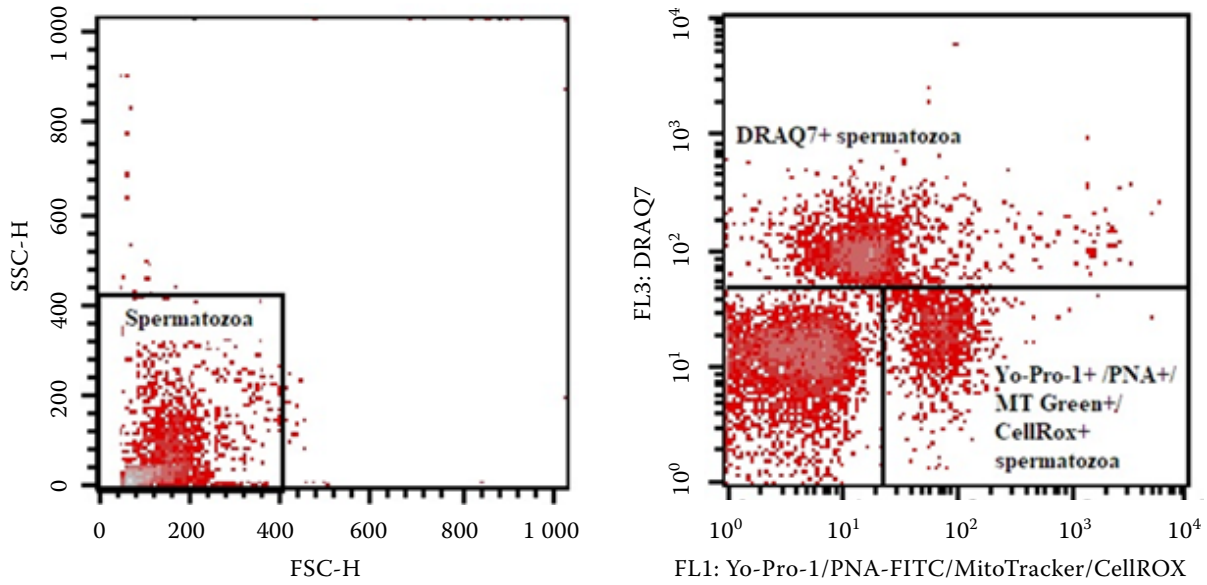

Figure 1. Flow cytometric gating strategy

Illustrative dot plots used for the flow cytometric evaluation of the rabbit spermatozoa

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Table 1. Effect of ellagic acid on motility parameters of rabbit thawed semen

Motility parameter	Ellagic acid concentration (mM)			
	0	0.5	1.5	2.5
Total motility (%)	45.2 ± 3.38	43.9 ± 4.77	49.6 ± 5.27	48.1 ± 2.93
Progressive motility (%)	28.6 ± 2.72	32.5 ± 5.19	32.2 ± 2.13	34.4 ± 1.93
DCL (μm/s)	33.9 ± 5.53	39.4 ± 6.76	35.8 ± 5.71	41.2 ± 6.52
VAP (μm/s)	40.4 ± 4.25	45.2 ± 6.00	43.2 ± 6.22	48.6 ± 6.06
VCL (μm/s)	76.1 ± 10.49	88.0 ± 13.5	78.0 ± 11.7	92.0 ± 13.1
VSL (μm/s)	30.6 ± 4.00	36.0 ± 4.78	33.5 ± 5.22	38.8 ± 5.81
STR (%)	0.75 ± 0.02	0.79 ± 0.00	0.77 ± 0.02	0.79 ± 0.02
LIN (%)	0.40 ± 0.01	0.41 ± 0.02	0.42 ± 0.01	0.42 ± 0.02
WOB (%)	0.53 ± 0.02	0.52 ± 0.02	0.56 ± 0.02	0.53 ± 0.02
ALH (μm/s)	3.67 ± 0.12	3.72 ± 0.24	3.36 ± 0.28	3.92 ± 0.20

ALH = mean amplitude of the lateral head displacement; DCL = distance curve line; LIN = linearity; STR = straightness; VAP = average path velocity; VCL = curvilinear velocity; VSL = straight-line velocity; WOB = wobble

Table 2. Effect of ellagic acid on the spermatozoa traits of rabbit thawed semen assessed by flow cytometry

Parameter	Ellagic acid concentration (mM)			
	0	0.5	1.5	2.5
Dead spermatozoa (%)	46.1 ± 3.62	45.3 ± 2.67	42.7 ± 3.90	44.2 ± 4.53
Apoptotic spermatozoa (%)	35.0 ± 1.72	32.5 ± 3.69*	17.2 ± 2.30*	26.7 ± 4.24
Acrosome integrity (%)	17.4 ± 1.78	13.1 ± 1.10	14.0 ± 1.68	15.0 ± 2.30
Mitochondrial activity (%)	29.9 ± 2.61	33.3 ± 2.40	34.7 ± 1.82	27.7 ± 2.89
ROS production (%)	25.0 ± 3.00	12.1 ± 0.80*	12.0 ± 0.67*	16.8 ± 1.70*

*Significant differences compared to the control at $P < 0.05$

apoptotic spermatozoa (Yo-Pro-1+), acrosome integrity (PNA+), mitochondrial activity (MT Green+), and ROS production (CellRox+). Based on the obtained results, none of the EA doses demonstrated a statistically significant impact on the proportion of deceased cells. Similarly, acrosome integrity and mitochondrial activity of spermatozoa remained unaffected by varying EA concentrations.

An important observation was the considerable reduction in ROS formation within spermatozoa across all groups receiving EA, in comparison with the control group ($P \leq 0.05$). Additionally, the supplementation of EA to the cryopreservation medium at a concentration of 1.5 mM resulted in a significant decrease ($P \leq 0.05$) in apoptosis when compared to both the control group and the EA1 group.

DISCUSSION

The sperm has a well-defined antioxidant system to combat oxidative stress, but during the process of dilution, cooling and freezing, the antioxidant power against ROS decreases (Ahmad et al. 2021). In this sense, the aim of this study was to identify the influence of ellagic acid, as a substance with antioxidant properties, on the quality of rabbit spermatozoa after cryopreservation.

As far as we know, several studies have been conducted using ellagic acid as a supplement to the sperm of different animal species and humans (Omur and Coyan 2016; Kia et al. 2017; Bucak et al. 2019; Najafi et al. 2019; Mottola et al. 2022). We observed that the values of total and progressive sperm motility did not change after the addition of EA to sperm, which is consistent with the re-

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search of Mottola et al. (2022) on human sperm, where EA did not cause any changes in sperm parameters. The effects of EA on the kinematic parameters were not significant ($P > 0.05$). However, it does not agree with previous studies on different animal species (Kia et al. 2017; Bucak et al. 2019; Najafi et al. 2019).

Omur and Coyan (2016) reported that the freezing of ram sperm with an extender supplemented with 1 or 2 mM EA resulted in higher sperm motility after thawing compared to the control or to the study of Najafi et al. (2019) on rooster sperm. Bucak et al. (2019) reported the best results in ram sperm obtained using 2 mM of EA compared to the control.

In our study on rabbit ejaculate, we found through the flow cytometry analysis that EA helped preserve some sperm parameters during storage in liquid nitrogen that are important for the fertilizing ability of the sperm. EA, given at 1.5 mM, reduced the occurrence of apoptotic sperm (Yo-Pro-1) to 17.2% compared to 35% in the control. Our results agree with the report of Ceribasi et al. (2012), who showed the effects of EA on ameliorating high levels of adriamycin-induced LPO and apoptosis in rats. Also, in the study by Najafi et al. (2019) apoptosis was prevented, when 1 mM of EA was used to freeze rooster sperm.

The acrosome integrity and mitochondrial activity were not affected by any concentration of EA. This is not in accordance with the research of Omur and Coyan (2016), where the addition of ellagic acid to frozen ram sperm improved membrane function and acrosomal integrity after thawing compared to the untreated control.

Regarding the production of ROS in rabbit sperm, the addition of EA at a concentration of 1.5 mM was the best compared to the control. This coincides with the results of several studies on bird sperm, where extenders supplemented with antioxidants were useful for inhibiting the generation of ROS (Fattah et al. 2017; Lotfi et al. 2017).

The positive effects of ellagic acid may be due to its antioxidant properties. Ceribasi et al. (2012) indicated that this polyphenol not only exhibits antioxidant and antiapoptotic properties, but also it can chelate metal ions and, therefore, prevent iron- and copper-catalyzed ROS generation. These and other authors reported a protective effect, when rats were treated with chemo-toxic compounds (Turk et al. 2008; Ceribasi et al. 2012; Rostami et al. 2022). Cisplatin induced incremental abnormalities in rat sperm which could be blocked by ellagic acid (Turk et al. 2008). Similarly, the cyclophosphamide induced lipid peroxidation leading to structural damage to spermatozoa and the testicular tissue of rats. Ellagic acid (2 mg/kg) also exerted a protective effect in this scenario (Ceribasi et al. 2010).

Cryopreservation may lead to the formation of ROS and to the reduction of antioxidant levels. ROS ultimately lead to a loss of membrane integrity, reduced sperm motility, leakage of intracellular enzymes and sperm DNA damage through oxidative stress and production of cytotoxic aldehydes (Baspinar et al. 2011) resulting in lower fertility.

Our study contradicts the findings of Baumber et al. (2005), who showed a significant decrease in equine sperm motility associated with ROS. This study contrasted with those indicating that supplementation of antioxidants to boar and dog semen increased sperm motility through prevention of ROS generation (Cerolini et al. 2000; Michael et al. 2009). Different observations in the sensitivity of sperm to oxidative stress may be due to differences in experimental methodology and animal species.

CONCLUSION

Based on the current findings, it is concluded that the addition of EA to extenders has a beneficial impact on rabbit spermatozoa apoptosis and ROS levels during cryopreservation. A dose of 1.5 mM is identified as the most effective for safeguarding spermatozoa during this process. However, further studies are essential to substantiate the positive effect of EA on fertility rates, both *in vitro* and *in vivo*, particularly in rabbits inseminated with frozen-thawed semen.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Ahmad E, Naseer Z, Aksoy M. Glutathione supplementation in semen extender improves rabbit sperm attributes during refrigeration. World Rabbit Sci. 2021 Jun 30;29(2):81-6.

https://doi.org/10.17221/142/2023-CJAS

Atessahin A, Turk G, Karahan I, Yilmaz S, Ceribasi AO, Bulmus O. Lycopene prevents adriamycin-induced testicular toxicity in rats. Fertil Steril. 2006 Apr;85 Suppl 1:1216-22.

Baspinar N, Coyan K, Bucak MN, Tuncer PB. Effects of dithioerythritol on ram semen after the freeze-thawing process. Cryobiology. 2011 Dec;63(3):152-6.

Baumber J, Ball BA, Linfor JJ. Assessment of the cryopreservation of equine spermatozoa in the presence of enzyme scavengers and antioxidants. Am J Vet Res. 2005 May; 66(5):772-9.

Bucak MN, Bodu M, Baspinar N, Gungor S, Ili P, Acibaeva B, Topraggaleh TR, Dursun S. Influence of ellagic acid and ebselen on sperm and oxidative stress parameters during liquid preservation of ram semen. Cell J. 2019 Apr;21(1):7-13.

Ceribasi AO, Turk G, Sonmez M, Sakin F, Atessahin A. Toxic effect of cyclophosphamide on sperm morphology, testicular histology and blood oxidant-antioxidant balance, and protective roles of lycopene and ellagic acid. Basic Clin Pharmacol Toxicol. 2010 Sep;107(3):730-6.

Ceribasi AO, Sakin F, Turk G, Sonmez M, Atessahin A. Impact of ellagic acid on adriamycin-induced testicular histopathological lesions, apoptosis, lipid peroxidation and sperm damages. Exp Toxicol Pathol. 2012 Nov;64 (7-8):717-24.

Cerolini S, Maldjian A, Surai P, Noble R. Viability, susceptibility to peroxidation and fatty acid composition of boar semen during liquid storage. Anim Reprod Sci. 2000 Feb 28;58(1-2):99-111.

Fattah A, Sharafi M, Masoudi R, Shahverdi A, Esmaeili V, Najafi A. L-Carnitine in rooster semen cryopreservation: Flow cytometric, biochemical and motion findings for frozen-thawed sperm. Cryobiology. 2017 Feb;74:148-53.

Hassoun EA, Walter AC, Alsharif NZ, Stohs SJ. Modulation of TCDD-induced fetotoxicity and oxidative stress in embryonic and placental tissues of C57BL/6J mice by vitamin E succinate and ellagic acid. Toxicology. 1997 Dec 19;124(1):27-37.

Hassoun EA, Vodhanel J, Abushaban A. The modulatory effects of ellagic acid and vitamin E succinate on TCDD-induced oxidative stress in different brain regions of rats after subchronic exposure. J Biochem Mol Toxicol. 2004;18(4):196-203.

Hassoun EA, Vodhanel J, Holden B, Abushaban A. The effects of ellagic acid and vitamin E succinate on antioxidant enzymes activities and glutathione levels in different brain regions of rats after subchronic exposure to TCDD. J Toxicol Environ Health A. 2006 Mar;69(5):381-93.

Holt WV. Fundamental aspects of sperm cryobiology: The importance of species and individual differences. Theriogenology. 2000 Jan 1;53(1):47-58.

Kia HD, Bolooki Z, Dodaran HV, Mahdipour M. Effect of adding coenzyme Q10 and ellagic acid during cryopreservation on post thaw quality of ram semen. Iranian J Appl Anim Sci. 2017;7(3):445-51.

Krockova J, Roychoudhury S, Slanina T, Formicki G, Binkowski LJ, Ondruska L, Lukac N, Kovacova R, Stawarz R, Massanyi P. Lead induced alterations in rabbit spermatozoa motility and morphology in vitro. Czech J Anim Sci. 2016;61(9):391-406.

Kubovicova E, Makarevich AV, Balazi A, Vasicek J, Chrenek P. Factors affecting rabbit sperm cryopreservation: A mini-review. Zygote. 2021 Jun 3;30(1):1-8.

Kulikova B, Balazi A, Supuka P, Chrenek P. Quality of fresh and frozen-thawed semen from Slovak native rabbit and it storage in the gene bank. Slovak J Anim Sci. 2017; 50(3):107-11.

Lotfi S, Mehri M, Sharafi M, Masoudi R. Hyaluronic acid improves frozen-thawed sperm quality and fertility potential in rooster. Anim Reprod Sci. 2017 Sep;184:204-10.

Michael AJ, Alexopoulos C, Pontiki EA, Hadjipavlou-Litina DJ, Saratsis P, Ververidis HN, Boscos CM. Effect of antioxidant supplementation in semen extenders on semen quality and reactive oxygen species of chilled canine spermatozoa. Anim Reprod Sci. 2009 May;112(1-2):119-35.

Moce E, Vicente JS. Rabbit sperm cryopreservation: A review. Anim Reprod Sci. 2009 Jan;110(1-2):1-24.

Mottola F, Iovine C, Carannante M, Santonastaso M, Rocco L. In vitro combination of ascorbic and ellagic acids in sperm oxidative damage inhibition. Int J Mol Sci. 2022 Nov 25;23(23): 10 p.

Najafi A, Taheri RA, Mehdipour M, Martinez-Pastor F, Rouhollahi AA, Nourani MR. Improvement of post-thawed sperm quality in broiler breeder roosters by ellagic acid-loaded liposomes. Poult Sci. 2019 Jan 1;98(1):440-6.

Omur AD, Coyan 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;61(1):10-6.

Rostami A, Vakili S, Koohpeyma F, Jahromi BN, Aghajari ZA, Mahmoudikohani F, Saki F, Mahmoodi M, Jaberi KR, Movahedpour A, Khorchani MJ, Noroozi S. Ellagic acid effects on testis, sex hormones, oxidative stress, and apoptosis in the relative sterility rat model following busulfan administration. BMC Complement Med Ther. 2022 Jun 23;22(1): 14 p.

Sikka SC. Oxidative stress and role of antioxidants in normal and abnormal sperm function. Front Biosci. 1996 Aug 1;1:e78-86.

Turk G, Atessahin A, Sonmez M, Ceribasi AO, Yuce A. Improvement of cisplatin-induced injuries to sperm quality,

https://doi.org/10.17221/142/2023-CJAS

the oxidant-antioxidant system, and the histologic structure of the rat testis by ellagic acid. Fertil Steril. 2008 May;89(5 Suppl):1474-81.

Turk G, Ceribasi AO, Sakin F, Sonmez M, Atessahin A. Antiperoxidative and anti-apoptotic effects of lycopene and ellagic acid on cyclophosphamide-induced testicular lipid peroxidation and apoptosis. Reprod Fertil Dev. 2010a; 22(4):587-96.

Turk G, Sonmez M, Ceribasi AO, Yuce A, Atessahin A. Attenuation of cyclosporine A-induced testicular and spermatozoal damages associated with oxidative stress by ellagic acid. Int Immunopharmacol. 2010b Feb;10(2):177-82.

Vasicek J, Balazi A, Svoradova A, Vozaf J, Dujickova L, Makarevich AV, Bauer M, Chrenek P. Comprehensive flow-cytometric quality assessment of ram sperm intended for gene banking using standard and novel fertility biomarkers. Int J Mol Sci. 2022 May 25;23(11): 29 p.

Vizzarri F, Palazzo M, Casamassima D, Ondruska L, Massanyi M, Tirpak F, Formicki G, Gren A, Massanyi P. Lippia citriodora (verbascoside) extract supplementation: Effect on rabbit semen quality in vivo and in vitro. Czech J Anim Sci. 2019;64(1):1-10.

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