Contemporary Agriculture, 75(1): 37-49, 2026.
DOI: https://doi.org/10.2478/contagri-2026-0003
eISSN 2466-4774; ISSN 0350-1205

Research article

Effects of Resveratrol and Epicatechin on Oxidative Damage and Bacterial Contamination in Boar Semen Following Short-Term Storage

Filip Benko[1*], Michal Ďuračka[2], Ondřej Bučko[3], Miroslava Kačániová[4,5], Eva Tvrdá[1,6]

[1]Institute of Biotechnology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
[2]AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
[3]Institute of Animal Husbandry, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
[4]Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
[5]School of Medical & Health Sciences, University of Economics and Human Sciences in Warsaw, Okopowa 59, 01 043 Warszawa, Poland
[6]Chair of Animal Breeding and Biotechnology, Institute if Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 62, 51006 Tartu, Estonia
**Corresponding author*: filip.benko@uniag.sk

Submitted: 3.10.2025.
Reviewed: 19.12.2025.
Accepted: 29.1.2026.

ABSTRACT

The aim of this study was to evaluate the impact of natural polyphenols, resveratrol (RES) and epicatechin (EPI), on the oxidative balance, bacterial contamination and functionality of boar spermatozoa during short-term storage at 4 °C. Both biomolecules exhibit strong antioxidant and antimicrobial effects. Semen samples were collected from 15 adult Duroc boars and diluted in the Androhep Plus extender enriched with varying concentrations of RES or EPI (10, 25 and 50 µM). Selected parameters, including motility, mitochondrial activity, DNA fragmentation, protein oxidation (PO), lipid peroxidation (LPO) as well as the generation of reactive oxygen species (ROS) were analyzed after 24, 48 and 72 h. Exposure to 10 and 25 µM of RES significantly preserved motility (P<0.0001), increased mitochondrial function (P<0.0001), acrosome integrity (P<0.0001), and reduced the generation of ROS (P<0.0001), superoxide radicals (P<0.001, P<0.01) and LPO (P<0.001) after 24-72 h compared to the untreated control. In contrast, 50 µM of RES significantly decreased sperm motility (P<0.0001), membrane integrity (P<0.001), acrosome integrity (P<0.0001) as well as microbial growth (P<0.01). Following EPI treatment, 25 and 50 µM concentrations significantly preserved motility (P<0.0001), reduced ROS (P<0.0001; P<0.001), LPO (P<0.001) and superoxide production (P<0.001; P<0.01) compared to the control. Mitochondrial activity (P<0.0001), acrosome integrity (P<0.0001; P<0.001) and membrane integrity (P<0.0001) significantly increased in the groups enriched with 25 or 50 µM of EPI after 24-72 h. Furthermore, a significant decline (P<0.05) in microbial growth was observed in the group with the highest dose of EPI compared to the untreated group. Our findings suggest that RES and EPI may improve the quality of extended boar spermatozoa and exhibit antioxidant and antimicrobial properties. However, the application of both biomolecules depends on the dose and the duration of exposure.

Key words:
antioxidants, oxidative stress, boar, storage, bacteria

Abbreviations:
AI – artificial insemination; DNPH – 2,4-dinitrophenylhydrazine; ECG – epicatechin gallate; EGCG – epigallocatechin gallate; EPI – epicatechin; FeAA – ferrous ascorbate; LPO – lipid peroxidation; MDA – malondialdehyde; MOT – motility; PUFAs – polyunsaturated fatty acids; RES – resveratrol; ROS – reactive oxygen species

Citation:
Benko, F., Ďuračka, M., Bučko, O., Kačániová; M. & Tvrdá, E. 2026. Effects of Resveratrol and Epicatechin on Oxidative Damage and Bacterial Contamination in Boar Semen Following Short-Term Storage. *Contemporary Agriculture*, 75(1): 37-49. https://doi.org/10.2478/contagri-2026-0003

INTRODUCTION

Artificial insemination (AI) is the major technique applied in the pig breeding industry, accounting for over 90% of all piglets in the global pork production. The primary benefit of AI lies in efficient dissemination of boar semen to a large population of sows, leading to overall genetic improvement and transmission of quality genes to the next generation (Gadea, 2003; Knox, 2016). In boars, AI relies on liquid-stored semen due to its superior fertility outcomes compared to frozen-thawed semen, which typically exhibits much lower quality (Rodríguez-Gil and Estrada, 2013).

Despite significant advances in cryopreservation techniques, liquid preservation remains the main method to preserve boar semen until insemination, since boar spermatozoa are highly sensitive to temperature fluctuations. Semen extenders and diluents are the primary media used for the liquid preservation of boar spermatozoa, supplying vital nutrients to support sperm metabolism, cold shock protectants, substances that help regulate pH and osmotic balance, as well as antibiotics to prevent potential microbial growth. In addition, semen extenders should protect spermatozoa against possible toxic effects of seminal plasma by reducing the metabolic rate in refrigerated boar semen. A higher content of polyunsaturated fatty acids (PUFAs) in the sperm plasmatic membrane, combined with limited intracellular antioxidant defenses, makes boar spermatozoa particularly vulnerable to oxidative stress, which could subsequently lead to the loss of viable gametes (Gadea, 2003; Teixeira et al., 2015; Peña et al., 2017; Waberski et al., 2019).

Oxidative damage to spermatozoa primarily arises from an imbalance between reactive oxygen species (ROS) generation and intracellular free radical-scavenging activities. Under physiological conditions, the ROS-opposing potential of the ejaculate is secured by antioxidants (vitamin E, vitamin C, glutathione, etc.) present in the seminal plasma. Nevertheless, when ejaculated semen is collected and processed, the levels of the antioxidant machinery rapidly decrease. Such conditions compromise the integrity of sperm membranes, ultimately reducing sperm motility, viability and fertilization ability. To counteract these negative effects, the addition of antioxidants to commercially used semen extenders has been suggested as a strategy to improve sperm quality and minimize ROS-induced damage during cooling and *in vitro* storage (Aitken, 1995; Michael et al., 2009; Vongpralub et al., 2016).

Resveratrol (RES) is a naturally occurring nonflavonoid polyphenol found in various plants, particularly in grapes and berries. It possesses strong antioxidant, antibacterial and antifungal effects. RES functions by neutralizing free radicals, including superoxide anion, hydroxyl radical and metal-induced reactive species. This natural compound also exhibits defensive effects against LPO and sperm DNA fragmentation (Branco et al., 2010; Jasiński et al., 2013; Bucak et al., 2014).

Catechin and epicatechin (EPI) are polyphenolic compounds (flavonols) belonging to the flavonoid family, which are capable of interacting with cellular membranes and incorporating into lipid bilayers. EPI is a stereoisomer of catechin and can be found mainly in green tea. Catechins have antioxidant properties, which include the ability to scavenge ROS, chelate metal ions and stimulate antioxidant enzymes (Fraga et al., 2010; Braicu et al., 2013; Bernatoniene and Kopustinskiene, 2018). The objective of our research was to investigate the potential antioxidant and antimicrobial effects and subsequently to determine the specific doses of RES and EPI effective in the reduction of the oxidative damage and bacterial contamination affecting boar spermatozoa during short-term storage.

MATERIAL AND METHODS

Sample treatment and storage

Ejaculates were collected from 15 sexually mature Duroc boars (Farm Terezov, Hlohovec, Slovak Republic). The obtained samples were kept at laboratory temperature (20-22°C) for 1 h. Each sample was diluted in a 1:20 ratio with the Androstar Plus^{TM} extender (Minitüb GmbH, Tiefenbach, Germany) without antibiotics. Experimental groups, except for the control, were supplemented with selected concentrations (10, 25 and 50 µM) of RES and EPI, dissolved in 0.5% dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA) and analyzed after 24, 48 and 72 h at 4°C. The control group contained the same final concentration of DMSO to exclude solvent-related effects.

Motility evaluation
Sperm motility rate (MOT, %) was examined using computer-assisted semen analysis (CASA; Version 14.0 TOX IVOS II; Hamilton-Thorne Biosciences, Beverly, USA) according to the protocol of Ďuračka et al. (2019).

Mitochondrial toxicity test
For the measurement of the mitochondrial activity, the mitochondrial toxicity test (MTT test) was used. This assay is based on the colorimetric change of a yellow tetrazolium salt to blue formazan, which indicates the activity of mitochondrial succinate dehydrogenase. The rate of optical density was measured spectrophotometrically using the Glomax Multi$^+$ (Promega, Madison, WI, USA) at 570 nm against 620 nm (Ďuračka et al., 2019).

Superoxide radical test
The concentration of the superoxide radical was determined using the nitroblue-tetrazolium (NBT) assay. Superoxide radicals present in the cells react with yellow nitroblue tetrazolium chloride, resulting in the formation of formazan crystals. The intensity of the formazan formation was quantified with the Glomax Multi$^+$ combined spectro-fluoro-luminometer (Promega, Madison, WI, USA) at 570 nm against 620 nm.

Sperm viability and acrosome integrity test
The level of membrane and acrosome integrity was examined using Trypan blue/Giemsa staining, which stains the sperm cell membranes as well as acrosomes. Based on the staining results, we evaluated different patterns: (1) acrosome-intact live/dead; (2) acrosome-lost live/dead. Live spermatozoa were characterized by a viable head and tail compared to dead cells (Boccia et al., 2007).

DNA fragmentation test
The integrity of DNA was observed by the Halomax Diagnostic kit (Halotech, Madrid, Spain) for boar spermatozoa. Damaged sperm cells are characterized by the formation of fragmented DNA loops in the head, which are visible under a fluorescent microscope (Leica, Holzheim, Germany) at 400x magnification (Halotech, Madrid, Spain).

Reactive oxygen species generation
The overall generation of ROS in semen was quantified by the chemiluminescence method, which uses luminol (Sigma-Aldrich, USA) as a probe. The rates of chemiluminescence following the reaction of ROS with luminol were evaluated on 96-well plates with the Glomax Multi$^+$ (Promega, Madison, WI, USA); (Ďuračka et al., 2016).

Protein oxidation test
A common 2,4-dinitrophenylhydrazine (DNPH) method, modified by Weber et al. (2015), was used for the assessment of the carbonyl groups. Samples were added to DNPH, then mixed and incubated with trichloroacetic acid (Sigma-Aldrich). The supernatant was removed and samples were washed in ethanol/ethyl acetate to remove the residual DNPH. Following treatment with guanidine-hydrochloric acid (Sigma-Aldrich), the samples were measured at 360 nm using a spectrophometer (Cary 60 UV-Vis, Agilent, USA); (Tvrdá et al., 2021).

Lipid peroxidation
For the evaluation of lipid peroxidation (LPO), we applied the TBARS (thiobarbituric acid reactive substances) assay adapted for 96-well plates and an ELISA reader, which quantified the amount of malondialdehyde (MDA) as the secondary product of LPO. The ultimate concentration of MDA was measured with the Glomax Multi$^+$ (Promega, Madison, WI, USA) at 540 nm (Tvrdá et al., 2016).

Bacterial identification
For the identification of bacteria in boar semen, 100 µl of each sample was inoculated and cultured on sterile blood, Gassner, MacConkey or Tryptic soy agar (MHB, Oxoid, Basingstoke, UK) under aerobic conditions at 37 °C for 24-48 h. Following cultivation, bacterial counts were determined as colony-forming units (CFU/mL) and colonies were purified by the four-quadrant streaking method (Ďuračka et al., 2021). The purified colonies were identified by the MALDI-TOF Biotyper mass spectrometry (Brucker Daltonics, Bremen, Germany), with the resulting spectra analyzed against the MALDI Biotyper Bruker Taxonomy database (Bruker Daltonics, Bremen, Germany), (Tvrdá et al., 2021).

Statistical analysis

Data analysis was performed using the GraphPad Prism program (version 6.02 for Windows; GraphPad Software, La Jolla, CA, USA, http://www.graphpad.com). Statistical comparisons were conducted using one-way ANOVA, followed by Dunnett's multiple comparison test based on comparisons between the control group and the experimental groups. The level of significance was indicated as follows: $^{****}P<0.0001$; $^{***}P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$.

RESULTS

The results of this study suggest that the impact of both bioactive compounds on boar spermatozoa is influenced by the dose and the duration of exposure. Analysis of motility (Table 1) revealed that the highest concentration of RES (50 µM) led to a significant decline (P<0.0001; P<0.05) in motility after 48 and 72 h when compared to the control. On the other hand, lower concentrations (10 and 25 µM) of RES preserved (P<0.0001) motility in all time periods of storage. In the case of EPI, motility was significantly higher (P<0.0001) in all tested concentrations compared to the control; however, the most pronounced effect was observed at 50 µM of EPI across all time periods.

Table 1. Effects of selected concentrations of resveratrol and epicatechin on boar sperm motility following 24-72 h of semen storage

Sperm motility [%]		**24 h**	**48 h**	**72 h**
Control		55.33±2.60	55.00±3.46	44.33±3.48
Resveratrol	**10 µM**	87.33±3.71****	85.67±5.69****	83.00±4.73***
	25 µM	87.33±3.84****	81.67±2.33****	69.67±10.09*
	50 µM	45.00±2.89	27.67±1.86****	21.00±2.08*
Epicatechin	**10 µM**	87.67±3.38****	82.00±3.17****	75.67±2.96**
	25 µM	87.00±0.57****	80.67±2.03****	77.00±6.93**
	50 µM	90.33±1.45****	86.33±1.45****	85.67±4.33***

Legend: Mean±SEM, $^{****}P<0.0001$; $^{***}P<0.001$; $^{**}P<0.01$; $^{*}P<0.05$.

The highest RES dose (50 µM) resulted in a significant reduction (P<0.001; P<0.0001) in mitochondrial activity (Table 2) following 72 h of cultivation. Similarly to the motility, lower RES doses (10 and 25 µM) significantly (P<0.001; P<0.0001) improved mitochondrial activity against the control in all time periods. All tested concentrations of EPI resulted in a significant increase in mitochondrial activity (P<0.001; P<0.0001) following 24 – 72 h of storage.

Table 2. Effects of selected concentrations of resveratrol and epicatechin on boar sperm mitochondrial activity following 24-72 h of semen storage

Mitochondrial activity [%]		**24 h**	**48 h**	**72 h**
Control		100.00±5.77	100.00±1.12	100.00±2.31
Resveratrol	**10 µM**	132.70±1.86***	141.70±2.19****	148.70±0.88****
	25 µM	123.30±7.69*	129.00±3.79**	128.00±4.36****
	50 µM	88.33±1.67	70.00±4.93***	53.00±4.93****
Epicatechin	**10 µM**	126.30±4.09**	133.70±4.09***	130.70±3.84****
	25 µM	128.00±3.79**	137.70±1.45****	148.70±0.88****
	50 µM	133.30±3.38***	143.30±4.41****	155.70±2.33****

Legend: Mean±SEM, $^{****}P<0.0001$; $^{***}P<0.001$; $^{**}P<0.01$; $^{*}P<0.05$.

Superoxide generation (Table 3) significantly decreased (P<0.01; P<0.001) in the groups supplemented with 10 and 25 µM RES in comparison with the control, except for the group containing 50 µM RES. The groups supplemented with EPI showed a significant superoxide reduction (P<0.01; P<0.001) already after 24 h. A decline in superoxide production was observed at all concentrations of EPI and all time periods.

Table 3. Effects of selected concentrations of resveratrol and epicatechin on superoxide production by boar spermatozoa following 24-72 h of semen storage

Superoxide production [%]		24 h	48 h	72 h
Control		100.00±5.77	100.00±2.31	100.00±1.12
Resveratrol	**10 µM**	81.67±2.91**	74.33±1.76***	70.00±3.79*
	25 µM	87.00±1.16*	79.67±0.88**	79.00±1.53
	50 µM	109.00±3.79	106.70±4.81	123.3±14.53
Epicatechin	**10 µM**	82.33±1.76**	83.67±4.91*	76.33±3.76
	25 µM	79.33±1.20***	77.33±3.48**	73.00±1.73*
	50 µM	77.67±1.76***	77.33±2.33**	67.00±4.58**

Legend: Mean±SEM, ****P<0.0001; ***P<0.001; **P<0.01; *P<0.05.

A significantly higher (P<0.0001) proportion of spermatozoa with an intact membrane (Table 4) was found in the groups treated with 10 or 25 µM of RES after 72 h when compared to the control. In contrast, exposure to 50 µM of RES during 48 and 72 h of storage resulted in a significantly higher proportion of membrane-damaged spermatozoa (P<0.001). The addition of all EPI consistently maintained a higher proportion (P<0.0001) of membrane-intact spermatozoa following 72 h of storage.

Table 4. Effects of selected concentrations of resveratrol and epicatechin on boar sperm membrane integrity following 24-72 h of semen storage

Membrane integrity		24 h	48 h	72 h
Control		86.67±1.45	76.33±3.18	55±1.73
Resveratrol	**10 µM**	93.00±1.73	89.33±0.33*	81.67±1.76****
	25 µM	90.67±2.73	84.00±2.52	78.00±1.53****
	50 µM	83.00±1.53	55.00±1.16***	36.33±2.40***
Epicatechin	**10 µM**	93.33±1.86	80.00±3.51	76.00±1.16****
	25 µM	94.67±0.88*	87.33±0.88*	77.33±1.20****
	50 µM	95.00±0.58*	89.84±1.22**	84.67±1.86****

Legend: Mean±SEM, ****P<0.0001; ***P<0.001; **P<0.01; *P<0.05.

Assessment of acrosome integrity (Table 5) revealed a significant improvement (P<0.0001) in the proportion of spermatozoa with intact acrosomes in the group supplemented with 10 µM RES following 48 and 72 h. A significant decrease (P<0.001; P<0.0001) in acrosome integrity was recorded in the presence of 50 µM RES, especially after 48 and 72 h. In the case of 50 µM EPI, our results indicate an increased (P<0.001) proportion of spermatozoa with preserved acrosomes during 48 and 72 h of storage.

Table 5. Effects of selected concentrations of resveratrol and epicatechin on boar sperm acrosome integrity following 24-72 h of semen storage

Acrosome integrity		24 h	48 h	72 h
Control		86.33±1.76	72±1.73	63.67±2.91
Resveratrol	**10 µM**	90.33±0.89	88.67±0.88****	79.67±0.88****
	25 µM	87.00±1.15	81.33±1.86**	74.67±1.20**
	50 µM	79.67±1.45*	56.33±1.85****	49.33±1.21***
Epicatechin	**10 µM**	88.67±0.88	77.33±1.45	74.67±1.20**
	25 µM	89.67±0.89	80.67±0.67**	71.67±1.76*
	50 µM	90.33±0.89	84.33±1.20***	75.67±2.60***

Legend: Mean±SEM, ****P<0.0001; ***P<0.001; **P<0.01; *P<0.05.

DNA fragmentation (Table 6) was affected by RES concentration and storage duration. While lower concentrations were associated with a significant decrease in DNA fragmentation after 24 h (P<0.001) and 72 h (P<0.01), exposure to 50 µM of RES resulted in a significant increase (P<0.05) in DNA fragmentation, most notably after 48 h of storage. The most pronounced decline was detected in the group enriched with 10 µM RES. In comparison to the control, exposure to 10-50 µM EPI led to a significantly reduced level (P<0.0001) of DNA damage after 24 h of storage.

Table 6. Effects of selected concentrations of resveratrol and epicatechin on boar sperm DNA damage following 24-72 h of semen storage

DNA damage		24 h	48 h	72 h
Control		15.87±0.45	18.53±0.78	24.15±1.29
Resveratrol	**10 µM**	11.01±0.12***	12.74±1.63*	15.94±1.01**
	25 µM	12.91±0.31*	14.95±1.07	19.67±0.39
	50 µM	17.53±0.51	23.67±2.03*	25.94±2.56
Epicatechin	**10 µM**	11.39±0.61***	15.96±0.89	19.11±0.68
	25 µM	10.00±0.58****	15.29±0.22	18.21±1.74*
	50 µM	9.07±1.07****	13.96±0.61	16.30±1.46**

Legend: Mean±SEM, ****P<0.0001; ***P<0.001; **P<0.01; *P<0.05.

Table 7 reveals that ROS generation increased continuously over the storage period in the control group. Significantly lower concentrations (P<0.0001; P<0.001) of ROS were recorded in the groups enriched with 10 and 25 µM RES following 48 and 72 h. EPI exhibited antioxidant effects during all time periods of storage by significantly decreasing (P<0.001; P<0.0001) global ROS generation, with the most pronounced scavenging effect observed at 25 and 50 µM of EPI.

Table 7. Effects of selected concentrations of resveratrol and epicatechin on reactive oxygen species (ROS) production by boar spermatozoa following 24-72 h of semen storage

ROS generation		24 h	48 h	72 h
Control		15.33±1.76	27.33±1.20	34.33±1.76
Resveratrol	**10 µM**	7.67±0.67**	15.00±1.73****	21.67±2.03***
	25 µM	9.60±0.62*	18.33±0.67***	24.00±1.53**
	50 µM	16.00±2.31	30.33±1.20	37.67±1.76
Epicatechin	**10 µM**	8.74±0.88**	17.00±1.17****	24.67±1.45**
	25 µM	8.35±0.57**	16.33±1.79****	23.33±0.88***
	50 µM	7.64±0.58**	14.01±1.21****	19.67±1.20****

Legend: Mean±SEM, ****P<0.0001; ***P<0.001; **P<0.01; *P<0.05.

According to the results of the protein oxidation assay (Table 8), a decrease in protein carbonyls was observed only in the group treated with 10 µM of RES after 72 h. Other tested doses showed non-significant changes compared to the control. EPI exhibited the most visible effects after 72 h of storage, where a significant decline in protein oxidation levels was noted in groups supplemented with 25 and 50 µM EPI compared to the control.

Table 8. Effects of selected concentrations of resveratrol and epicatechin on protein oxidation of boar spermatozoa following 24-72 h of semen storage

Carbonyl groups formation		24 h	48 h	72 h
Control		1.49±0.25	2.49±0.34	4.91±0.29
Resveratrol	**10 µM**	1.12±0.21	1.78±0.17	2.74±0.32**
	25 µM	1.46±0.21	2.12±0.26	4.62±0.074
	50 µM	2.23±0.19	3.23±0.54	5.52±0.61
Epicatechin	**10 µM**	1.17±0.22	1.60±0.31	3.07±0.62*
	25 µM	0.97±0.19	1.64±0.17	2.96±0.36**
	50 µM	0.98±0.11	1.45±0.19	2.85±0.18**

Legend: Mean±SEM, ****P<0.0001; ***P<0.001; **P<0.01; *P<0.05.

As Table 9 shows, the protective effects of RES were notable after 48 h of storage in the groups treated with 10 and 25 µM RES (P<0.001). After 24-72 h, the lipoprotective effects of EPI were detectable with a significant reduction (P<0.05; P<0.01; P<0.001; P<0.0001) in MDA levels following exposure of boar spermatozoa to all concentrations of EPI.

Table 9. Effects of selected concentrations of resveratrol and epicatechin on lipid peroxidation of boar spermatozoa following 24-72 h of semen storage

Lipid peroxidation		24 h	48 h	72 h
Control		8.85±0.32	14.45±0.25	20.18±0.15
Resveratrol	**10 µM**	7.47±0.17	9.14±0.11***	15.18±0.63**
	25 µM	7.69±0.49	9.83±0.23***	18.52±0.43
	50 µM	9.92±0.29	15.28±1.58	22.75±1.38
Epicatechin	**10 µM**	7.36±0.17*	10.17±0.17***	18.52±0.81
	25 µM	6.59±0.28**	9.947±0.35***	14.42±0.59***
	50 µM	6.41±0.11***	9.55±0.29***	13.53±0.78****

Legend: Mean ± SEM. ****P<0.0001; ***P<0.001; **P<0.01; *P<0.05; RES (Resveratrol); EPI (Epicatechin)

The presence of bacteria was detected in all tested samples. The predominant species included *Pseudomonas spp.*, *Clostridium spp.*, *Enterococcus spp.*, *Bacillus spp.* and *Corynebacterium spp.* Data presented in Table 10 showed that 25 µM RES effectively eradicated *Enterococcus* spp. after 48 h, while 50 µM RES had the same effect right after 24 h. Based on the bacterial count, there was a significant decrease (P<0.05; P<0.01) in bacterial presence in the groups supplemented with 25 and 50 µM RES in all time periods of storage (24-72 h) compared to the control.

Table 10. Bacterial profiles of extended boar semen supplemented with selected concentrations of resveratrol following 24-72 h of storage

Group	Storage duration	Identified bacteria and sample positivity	Bacterial count (log CFU/ml)
Ctrl	**24 h**	*P. aeruginosa* (20.00%), *P. vulgaris* (20.00%), *K. oxytoca* (20.00%), *P. mirabilis* (13.30%), *C. difficile* (13.30%), *E. hirae* (13.30%), *E. faecalis* (13.30%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *P. putida* (6.70%), *K. pneumoniae* (6.70%), *B. cereus* (6.70%), *A. iwoffii* (6.70%), *Corynebacterium spp.* (6.70%)	1.55±0.21
	48 h	*P. aeruginosa* (13.30%), *P. vulgaris* (13.30%), *K. oxytoca* (13.30%), *C. difficile* (13.30%), *E. hirae* (6.70%), *E. faecalis* (6.70%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *P. putida* (6.70%), *B. cereus* (6.70%), *Corynebacterium spp.* (6.70%)	0.97±0.35
	72 h	*P. aeruginosa* (6.70%), *P. vulgaris* (6.70%), *K. oxytoca* (6.70%), *C. difficile* (13.30%), *E. hirae* (6.70%), *E. faecalis* (6.70%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *B. cereus* (6.70%), *Corynebacterium spp.* (6.70%)	0.60±0.12
RES 10 µM	**24 h**	*P. aeruginosa* (20.00%), *P. vulgaris* (20.00%), *K. oxytoca* (20.00%), *P. mirabilis* (13.30%), *C. difficile* (13.30%), *E. hirae* (13.30%), *E. faecalis* (13.30%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *P. putida* (6.70%), *K. pneumoniae* (6.70%), *B. cereus* (6.70%), *A. iwoffii* (6.70%), *Corynebacterium spp.* (6.70%)	1.49±0.32
	48 h	*P. aeruginosa* (13.30%), *P. vulgaris* (13.30%), *K. oxytoca* (13.30%), *C. difficile* (13.30%), *E. hirae* (6.70%), *E. faecalis* (6.70%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *P. putida* (6.70%), *B. cereus* (6.70%), *Corynebacterium spp.* (6.70%)	0.90±0.17
	72 h	*P. aeruginosa* (6.70%), *P. vulgaris* (6.70%), *K. oxytoca* (6.70%), *C. difficile* (13.30%), *E. hirae* (6.70%), *E. faecalis* (6.70%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *B. cereus* (6.70%), *Corynebacterium spp.* (6.70%)	0.55±0.15
RES 25 µM	**24 h**	*P. aeruginosa* (20.00%), *P. vulgaris* (20.00%), *K. oxytoca* (20.00%), *P. mirabilis* (13.30%), *C. difficile* (13.30%), *E. hirae* (6.70%), *E. faecalis* (6.70%), *P. putida* (6.70%), *K. pneumoniae* (6.70%), *B. cereus* (6.70%), *A. iwoffii* (6.70%), *Corynebacterium*	1.01±0.19$^{*\text{Ctrl 24}}$

		spp. (6.70%)	
	48 h	*P. aeruginosa* (13.30%), *P. vulgaris* (13.30%), *K. oxytoca* (13.30%), *C. difficile* (13.30%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *P. putida* (6.70%), *B. cereus* (6.70%), *Corynebacterium spp.* (6.70%)	0.71±0.12$^{*Ctrl\ 48}$
	72 h	*P. aeruginosa* (6.70%), *P. vulgaris* (6.70%), *K. oxytoca* (6.70%), *C. difficile* (13.30%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *B. cereus* (6.70%), *Corynebacterium spp.* (6.70%)	0.40±0.10$^{*Ctrl\ 72}$
RES 50 µM	**24 h**	*P. aeruginosa* (20.00%), *P. vulgaris* (20.00%), *K. oxytoca* (20.00%), *P. mirabilis* (13.30%), *C. difficile* (13.30%), *P. putida* (6.70%), *K. pneumoniae* (6.70%), *A. iwoffii* (6.70%), *Corynebacterium spp.* (6.70%)	0.97±0.18$^{**Ctrl\ 24}$
	48 h	*P. aeruginosa* (13.30%), *P. vulgaris* (13.30%), *K. oxytoca* (13.30%), *C. difficile* (13.30%), *P. putida* (6.70%), *Corynebacterium spp.* (6.70%)	0.67±0.13$^{*Ctrl\ 48}$
	72 h	*P. aeruginosa* (6.70%), *P. vulgaris* (6.70%), *K. oxytoca* (6.70%), *C. difficile* (13.30%), *Corynebacterium spp.* (6.70%)	0.35±0.19$^{*Ctrl\ 72}$

In the case of epicatechin (Table 11), the highest dose of 50 µM successfully eradicated *Bacillus spp.* after 72 h of storage. There was also a visible significant reduction ($P<0.05$) in bacterial presence in the groups supplemented with 50 µM EPI following 24-72 h.

Table 11. Bacterial profiles of extended boar semen supplemented with selected concentrations of epicatechin following 24-72 h of storage

Group	**Storage duration**	**Identified bacteria and sample positivity**	**Bacterial count (log CFU/ml)**
Ctrl	**24 h**	*P. aeruginosa* (20.00%), *P. vulgaris* (20.00%), *K. oxytoca* (20.00%), *P. mirabilis* (13.30%), *C. difficile* (13.30%), *E. hirae* (13.30%), *E. faecalis* (13.30%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *P. putida* (6.70%), *K. pneumoniae* (6.70%), *B. cereus* (6.70%), *A. iwoffii* (6.70%), *Corynebacterium spp.* (6.70%)	1.55±0.21
	48 h	*P. aeruginosa* (13.30%), *P. vulgaris* (13.30%), *K. oxytoca* (13.30%), *C. difficile* (13.30%), *E. hirae* (6.70%), *E. faecalis* (6.70%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *P. putida* (6.70%), *B. cereus* (6.70%), *Corynebacterium spp.* (6.70%)	0.97±0.35
	72 h	*P. aeruginosa* (6.7%). *P. vulgaris* (6.7%). *K. oxytoca* (6.7%). *C. difficile* (13.3%). *E. hirae* (6.7%). *E. faecalis* (6.7%). *B. subtilis* (6.7%). *B. licheniformis* (6.7%). *B. cereus* (6.7%). *Corynebacterium spp.* (6.7%)	0.60±0.12
EPI 10 µM	**24 h**	*P. aeruginosa* (20.00%), *P. vulgaris* (20.00%), *K. oxytoca* (20.0%), *P. mirabilis* (13.30%), *C. difficile* (13.30%), *E. hirae* (13.30%), *E. faecalis* (13.30%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *P. putida* (6.70%), *K. pneumoniae* (6.70%), *B. cereus* (6.70%), *A. iwoffii* (6.70%), *Corynebacterium spp.* (6.70%)	1.51±0.16
	48 h	*P. aeruginosa* (13.30%), *P. vulgaris* (13.30%), *K. oxytoca* (13.30%), *C. difficile* (13.30%), *E. hirae* (6.70%), *E. faecalis* (6.70%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *P. putida* (6.70%), *B. cereus* (6.70%), *Corynebacterium spp.* (6.70%)	0.90±0.18
	72 h	*P. aeruginosa* (6.70%), *P. vulgaris* (6.70%), *K. oxytoca* (6.70%), *C. difficile* (13.30%), *E. hirae* (6.70%), *E. faecalis* (6.70%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *B. cereus* (6.70%), *Corynebacterium spp.* (6.70%)	0.55±0.11
EPI 25	**24 h**	*P. aeruginosa* (20.00%), *P. vulgaris* (20.00%), *K. oxytoca*	1.22±0.17

µM		(20.00%), *P. mirabilis* (13.30%), *C. difficile* (13.30%), *E. hirae* (13.30%), *E. faecalis* (6.70%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *P. putida* (6.70%), *K. pneumoniae* (6.70%), *B. cereus* (6.70%), *A. iwoffii* (6.70%), *Corynebacterium spp.* (6.70%)	
	48 h	*P. aeruginosa* (13.30%), *P. vulgaris* (13.30%), *K. oxytoca* (13.30%), *C. difficile* (13.30%), *E. hirae* (6.70%), *E. faecalis* (6.70%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *P. putida* (6.70%), *B. cereus* (6.70%), *Corynebacterium spp.* (6.70%)	0.81±0.25
	72 h	*P. aeruginosa* (6.70%), *P. vulgaris* (6.70%), *K. oxytoca* (6.70%), *C. difficile* (13.30%), *E. hirae* (6.70%), *E. faecalis* (6.70%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *Corynebacterium spp.* (6.70%)	0.47±0.10
EPI 50 µM	**24 h**	*P. aeruginosa* (13.30%), *P. vulgaris* (20.00%), *K. oxytoca* (20.00%), *P. mirabilis* (13.30%), *C. difficile* (13.30%), *E. hirae* (6.70%), *E. faecalis* (6.70%), *B. subtilis* (6.70%), *B. licheniformis* (6.70%), *P. putida* (6.70%), *K. pneumoniae* (6.70%), *B. cereus* (6.70%), *A. iwoffii* (6.70%), Corynebacterium *spp.* (6.70%)	1.00±0.24$^{*Ctrl\ 24}$
	48 h	*P. aeuginosa* (6.70%), *P. vulgaris* (13.30%), *K. oxytoca* (13.30%), *C. difficile* (13.30%), *E. hirae* (6.70%), *E. faecalis* (6.70%), *B. licheniformis* (6.70%), *P. putida* (6.70%), *B. cereus* (6.70%), *Corynebacterium spp.* (6.70%)	0.71±0.18$^{*Ctrl\ 48}$
	72 h	*P. vulgaris* (6.70%), *K. oxytoca* (6.70%), *C. difficile* (13.30%), *E. hirae* (6.70%), *E. faecalis* (6.70%), *B. licheniformis* (6.70%), *Corynebacterium spp.* (6.70%)	0.38±0.19$^{*Ctrl\ 72}$

DISCUSSION

Although currently available semen extenders are capable of preserving semen quality, their potential to reduce oxidative damage to spermatozoa remains limited. Oxidative stress (OS) is the main problem contributing to the deterioration of boar spermatozoa, which are very sensitive and vulnerable to the development of OS during storage (Peña et al., 2017). Despite the desirable preservation capacity of commercially available semen extenders, there is still space to improve their efficacy, by the addition of substances of natural origin with antioxidant properties. This experiment confirmed the potential beneficial antioxidant effects of RES and EPI on boar spermatozoa during short-term storage (24-72 hours), which led to a reduction of OS and an overall improvement in semen quality. However, our results showed that antioxidant efficiency depends on the dose and the duration of exposure.

Martin-Hidalgo et al. (2013) evaluated the effects of various RES concentrations (10, 33, 66 and 100 µM) on motility, viability and acrosome state of boar spermatozoa following short-term liquid storage for 7 days at 17°C. Higher concentrations of RES (100 µM) significantly increased the proportion of spermatozoa with dislocation of the membrane phospholipid layer and reduced the motility of boar spermatozoa. These results contribute to the theory that higher concentrations of RES may exert adverse effects on sperm cells and potentially compromise their fertilization ability. Conversely, lower doses of RES did not significantly impact spermatozoa viability or acrosomal integrity.

According to Chunrong et al. (2019), 50 µM RES improved post-thaw quality of goat semen. The authors observed a significant improvement ($P<0.05$) in total motility, membrane and acrosome integrity, and mitochondrial activity of buck spermatozoa. These findings may be attributed to species-specific differences, particularly in the composition of the sperm plasma membrane and the proportion of polyunsaturated fatty acids (PUFAs) present on the membrane surface in boars versus bucks.

Sun et al. (2020) demonstrated the antioxidant efficacy of higher doses of RES (50 and 150 µM) against oxidative damage after liquid preservation of boar spermatozoa in the Modena extender. RES notably improved ($P<0.05$) or preserved motility, increased the proportion of spermatozoa with intact acrosomes and cells with a higher mitochondrial potential. The production of ROS and levels of MDA were decreased after the addition of 50 µM of RES. However, our findings suggest that 50 µM of RES affected all parameters, particularly motility, mitochondria functionality and acrosome state, which led to a significant decrease in the quality of samples compared to the control. Such differences could be attributed to the use of different extenders in the experiments. Meanwhile, the

application of 50 µM RES enhanced the quality of thawed boar spermatozoa by increasing progressive motility, membrane and acrosome integrity, mitochondrial function and total antioxidant activity. An equal effect was observed in the production of ROS and lipid peroxidation, which were decreased in the group supplemented with 50 µM, RES (Zhu et al., 2019). Therefore, it may be hypothesized that higher RES concentrations may be more effective when spermatozoa are exposed to a harsher cryogenic environment associated with a more pronounced risk for sperm damage.

Similarly to our findings, the experiment of Nouri et al. (2018) confirmed the dose-dependent impact of RES and epigallocatechin-3-gallate (EGCG) on low-quality cryopreserved stallion semen samples. Lower RES doses (10 µM) and average doses of EGCG (50 µM) preserved the total motility of stallion spermatozoa after thawing. Moreover, these concentrations of RES and EGCG significantly improved ($P<0.05$) viability and membrane integrity, mitochondrial function, and decreased DNA fragmentation and LPO levels. In contrast, the highest RES concentrations (20 µM) had the opposite effects on all parameters and decreased the quality of post-thaw semen samples.

Green tea contains an appreciable amount of EPI, which has powerful antioxidant properties and the ability to prevent oxidative damage. The main findings by Gale et al. (2015) indicated that green tea added to the freezing extender decreased the level of LPO, although no improvement in the overall quality of thawed boar spermatozoa was observed. We may speculate that the impact of EPI could be affected by the application of green tea extract instead of the pure biomolecule.

Effects of epigallocatechin gallate (EGCG), which belongs to the catechins family, on cryopreserved boar spermatozoa were described by Kaedei et al. (2012). Spermatozoa were incubated in an IVF medium enriched with different concentrations of EGCG, which significantly increased ($P<0.05$) motility, but viability remained unchanged. The co-incubation with EGCG also increased the penetrability of boar spermatozoa used for *in vitro* fertilization. These findings are in agreement with Gadani et al. (2017), who confirmed that administration of RES and EGCG to boar spermatozoa significantly increased ($P<0.01$) fertilization efficiency and sperm penetration rate, even though acrosome integrity and viability of cells remained unchanged compared to the control.

In another report, supplementing semen extenders with various concentrations of catechin (25, 50, 75 and 100 µM) significantly improved ($P<0.05$) the boar sperm quality and reduced the level of LPO after 24-72 h. Motility and viability of boar sperm cells were notably higher than in the control ($P<0.05$); however, acrosome integrity in the groups treated with catechin was lower compared to the control ($P<0.05$) (Boonsorn et al., 2010).

Positive properties of EPI supplementation on qualitative sperm parameters and oxidative profile agree are consistent with the findings of Tvrdá et al. (2019). EPI was capable of decreasing the oxidative damage and ROS production induced by ferrous ascorbate (FeAA). Higher concentrations of EPI improved the quality of fresh and cryopreserved bovine spermatozoa.

Based on the results from previous studies, we may hypothesize that the activity and antioxidant properties of bioactive molecules like RES are partly influenced by the extent of cellular damage and surrounding culture conditions. If the spermatozoa are subjected to negative factors, such as low temperatures during the freezing process or the presence of prooxidants, it is necessary to apply higher doses of bioactive molecules to effectively exert their effect. Additionally, the use of extenders could be beneficial, since extenders often contain numerous components that support sperm survival by providing protection and nutrition for the cells during cultivation or the freezing process.

Earlier studies (Arakawa et al., 2004; Navarro-Martínez et al., 2005) confirmed that higher concentrations of polyphenolic catechins from green tea, including epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), are able to inhibit bacterial presence by disrupting cytoplasmic bacterial membrane. Stapleton et al. (2008) reported the inhibitory potential of ECG against *Staphylococcus aureus* and demonstrated its ability to displace a macromolecule of lipoteichoic acid from the membrane bilayer, potentially increasing the susceptibility of staphylococci to β-lactam antibiotics. According to Escandón et al. (2016) the antibacterial effect of epicatechin against *Helicobacter pylori* was dose-dependent. During a 48 h cultivation, growth agar enriched with higher concentrations of epicatechin (from 0.8 to 1.0 mg/mL) successfully inhibited the growth of *H. pylori*. We observed a similar phenomenon, since the antimicrobial effect of catechins increased with higher concentrations and prolonged incubation time.

Resveratrol has been studied for its antimicrobial activity against a variety of gram-positive as well as gram-negative pathogens. The highest sensitivity among tested bacteria to resveratrol (50 and 100 µg/mL) was observed in the case of *Bacillus cereus*, *Staphylococcus aureus* and *Enterococcus faecalis*. Interestingly, increasing concentrations of resveratrol showed bacteriostatic activity against *Bacillus cereus* (Paulo et al., 2010), which corroborates our data and suggests that resveratrol may eradicate specific strains of bacteria like *Bacillus spp*. A possible mechanism of action could involve the reversible binding of resveratrol to ATP synthase and inhibition of oxidative phosphorylation (Vestergaard and Ingmer, 2019).

CONCLUSION

In conclusion, RES and EPI exhibited antioxidant effects and beneficial properties against oxidative damage during short-term storage and improved the quality of boar spermatozoa. However, the manifestation of their antioxidant activity depends on the dose and the duration of storage. RES concentrations of 10 and 25 µM and EPI concentrations of 25 and 50 µM demonstrated protective effects against oxidative damage caused by ROS generation and preserved the qualitative and quantitative parameters of boar spermatozoa. Therefore, the administration of RES and EPI into semen extenders may be recommended, as their supplementation can help preserve boar spermatozoa vitality and minimize the risks associated with oxidative stress and bacterial contamination.

Authors' Contribution: Conceptualization: E.T.; Methodology: E.T., M.K. and M.Ď.; Software: E.T.; Validation: E.T.; Formal Analysis: M.K. and E.T.; Investigation: F.B.; Resources: O.B. and M.Ď.; Data Curation: E.T. and M.K.; Writing – Original Draft Preparation: F.B.; Writing – Review & Editing: F.B. and E.T.; Visualization: F.B.; Supervision: E.T.; Project Administration: E.T.; Funding Acquisition: E.T. and M.K." All authors have read and agreed to the published version of the manuscript.

Funding: This research was financed by the Slovak Research and Development Agency grants. no. APVV-21-0095, APVV-20-0058 and by the VEGA grant no. 1/0067/24.

Data protection issues: All animals had cared under the ethical guidelines of the Slovak Animal Protection Regulation RD 377/12, conforming to the European Directive 2010/63/EU causing no harm or discomfort to animals.

Data availability statement: The data presented in this study are available upon request from the authors.

Authors' ORCID iDs: Filip Benko (https://orcid.org/0000-0001-9652-0446); Michal Ďuračka (https://orcid.org/0000-0002-2907-5909); Ondřej Bučko (https://orcid.org/0000-0001-6942-511X); Miroslava Kačániová (https://orcid.org/0000-0002-4460-0222); Eva Tvrdá (https://orcid.org/0000-0003-2895-1249).

Conflict of interest: The authors declare that they have no conflict of interest.

REFERENCES

Aitken, R.J. 1995. Free radicals, lipid peroxidation and sperm function. *Reproduction, Fertility and Development*, 7(4): 659-668. https://doi.org/10.1071/rd9950659

Arakawa, H., Maeda, M., Okubo, S. & Shimamura,T. 2004. Role of hydrogen peroxide in bacterial action of catechin. *Biological and Pharmaceutical Bulletin*, 27(3): 277-281. https://doi.org/10.1248/bpb.27.277

Bernatoniene, J. & Kopustinskiene, D.M. 2018. The Role of Catechins in Cellular Responses to Oxidative Stress. *Molecules*, 23(4): 965. https://doi.org/10.3390/molecules23040965

Boccia, L., Di Palo, R., De Rosa, A., Attanasio, L., Mariotti, E. & Gasparrini, B. 2007. Evaluation of buffalo semen by Trypan blue/Giemsa staining and related fertility in vitro. *Italian Journal of Animal Science*, 6(2): 739-742. https://doi.org/10.4081/ijas.2007.s2.739

Boonsorn, T., Kongbuntad, W., Narkkong, N.A. & Aengwanich, W. 2010. Effects of catechin addition to extender on sperm quality and lipid peroxidation in boar semen. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 7(3): 283-288.

Braicu, C., Ladomery, M.R., Chedea, V.S., Irimie, A. & Berindan-Neagoe, I. 2013. The relationship between the structure and biological actions of green tea catechins. *Food Chemistry*, 141: 3282-3289. https://doi.org/10.1016/j.foodchem.2013.05.122

Branco, C.S., Garcez, M.E., Pasqualotto, F.F., Erdtman, B. & Salvador, M. 2010. Resveratrol and ascorbic acid prevent DNA damage induced by craopreservation in human semen. *Cryobiology*, 60: 235-237. https://doi.org/10.1016/j.cryobiol.2009.10.012

Bucak, M.N., Ataman, M.B., Baspinar, N., Uysal, O., Taspinar, M., Bilgili, A., Öztürk, C., Güngör, S., Inanc, M.E., & Akal, E. 2014. Lycopene and resveratrol improve post-thaw sperm parameters: sperm motility, mitochondrial activity and DNA integrity. *Andrologia*, 47(5): 545-552. https://doi.org/10.1111/and.12301

Ďuračka, M., Belić, L., Tokárová, K., Žiarovská, J., Kačániová, M., Lukáč, N. & Tvrdá, E. 2021. Bacterial communities in bovine ejaculates and their impact on the semen quality. *Systems Biology in Reproductive Medicine*, 67(6): 438-449. https://doi.org/10.1080/19396368.2021.1958028

Ďuračka, M., Debacker, M., Bučko, O., Lukáč, N. & Tvrdá, E. 2019. The effect of kaempferol and naringenin may improve the in vitro quality of stored boar semen. *Journal of Central European Agriculture*, 20(4): 1069-1075. https://doi.org/10.5513/jcea01/20.4.2294

Ďuračka, M., Tvrdá, E., Halenár, M., Zbyňovská, K., Kolesár, E. & Lukáč, N. 2016. The impact of amygdalin on the oxidative profile of rabbit testicular tissue. *MendelNet*, 770-775.

Escandón, R.A., Del Campo, M., López-Solis, R., Obreque-Slier, E. & Toledo, H. 2016. Antibacterial effect of kaempferol and –(-)-epicatechin on Helicobacter pylori. *European Food Research and Technology*, 242: 1495-1502. https://doi.org/10.1007/s00217-016-2650-z

Fraga, C.G., Galleano, M., Verstraete, S.V. & Oteiza, P.I. 2010. Basic biochemical mechanisms behind the health benefits of polyphenols. *Molecular Aspects of Medicine*, 31: 435-445. https://doi.org/10.1016/j.mam.2010.09.006

Gadani, B., Bucci, D., Spinaci, M., Tamanini, C. & Galeati, G. 2017. Resveratrol and Epigallocatechin-3-gallate addition to thawed boar sperm improves in vitro fertilization. *Theriogenology*, 90: 88-93. https://doi.org/10.1016/j.theriogenology.2016.11.020

Gadea, J. 2003. Review: Semen extenders used in the artificial insemination of swine. *Spanish Journal of Agricultural Research*, 1(2): 17-27. https://doi.org/10.5424/sjar/2003012-17

Gale, I., Gil, L., Malo, C., González, N. & Martínez, F. 2015. Effect of Camelia sinensis supplementation and increasing holding time on quality of cryopreserved boar semen. *Andrologia*, 47(5): 505-512. https://doi.org/10.1111/and.12293

Chunrong, L., Allai, L., Gooquan, W., QionghuaI, H. & Guobo, Q. 2019. Improving the quality of cryopreserved goat semen with a commercial bull extender supplemented with resveratrol. *Animal Reproduction Science*, 208: 106127. https://doi.org/10.1016/j.anireprosci.2019.106127

Jasiński, M., Jasińska, L. & Ogrodowczyk, M. 2013. Resveratrol in prostate disease – a short review. *Central European Journal of Urology*, 66: 144-149. https://doi.org/10.5173/ceju.2013.02.art8

Kaedei, Y., Naito, M., Naoi, H., Sato, Y., Taniguchi, M., Tanihara, F., KikuchiI, K., Nagai, K. & Otoi, T. 2012. Effects of (-)-Epigalocatechin Gallate on the Motility and Penetrability of Frozen-Thawed Boar Spermatozoa Incubated in the Fertilization Medium. *Reproduction in Domestic Animals*, 47(6): 880-886. https://doi.org/10.1111/j.1439-0531.2012.01984.x

Knox, R.V. 2016. Artificial insemination in pigs today. *Theriogenology*, 85(1): 83-93. https://doi.org/10.1016/j.theriogenology.2015.07.009

Martin-Hidalgo, D., Hurtado De Llera, A., Henning, H., Wallner, U., Waberski, D., Bragado, M.J., Gil, M.C. & Garcia-Marin, L.J. 2013. The effect od Resveratrol on the Quality of Extended Boar Semen During Storage at 17°C. *The Journal of Agricultural Science*, 5(8): 231-242. https://doi.org/10.5539/jas.v5n8p231

Michael, A.J., Alexopoulos, C., Pontiki, E.A., Handjipavlou-Litina, D.J., Saratsis, P., Ververidis, H.N. & Boscos, C.M. 2009. Effect of antioxidant supplementation in semen extenders on semen quality and reactive oxygen species of cilled canine spermatozoa. *Animal Reproduction Science*, 112(1-2): 119-135. https://doi.org/10.1016/j.anireprosci.2008.04.007

Navarro-Martínez, M.D., Navarro-Perán, E., Cabezas-Herrera, J., Ruiz-Gómez, J., Garcáa-Cánovas, F. & Rodríguez-López, J.N. 2005. Antifolate activity of epigallocatechin against Stenotrophomas maltophila. *Antimicrobial Agents and Chemotherapy*, 49(7): 2914-2920. https://doi.org/10.1128/aac.49.7.2914-2920.2005

Nouri, H., Shojaeian, K., Samadian, F., Lee, S., Kohram, H. & Lee, J.K. 2018. Using Resveratrol and Epigallocatechin-3-Gallate to Improve Cryopreservation of Stallion Spermatozoa With Low Quality. *Journal of Equine Veterinary Science*, 70: 18-25. https://doi.org/10.1016/j.jevs.2018.07.003

Paulo, L., Ferreira, S., Gallardo, E., Queiroz, J.A. & Domingues, F. 2010. Antimicrobial activity and effects of resveratrol on human pathogenic bacteria. *World Journal of Microbiology and Biotechnology*, 26: 1533-1538. https://doi.org/10.1007/s11274-010-0325-7

Peña, S.J.T., Gummov, B., Parker, A.J. & Paris, D.B.B.P. 2017. Revisiting summer infertility in the pig: could heat stress induced sperm DNA damage negatively affect early embryo development? *Animal Production Science*, 57(10): 1975-1983. https://doi.org/10.1071/an16079

Rodríguez-Gil, J.E. & Estrada, E. 2013. Artificial insemination in boar reproduction. In: Bonet S, Casas I, Holt WV, Yeste M, editors. Boar reproduction. p. 589-608, Berlin, Springer.

Stapleton, P.D., Shah, S., Ehlert, K., Hara, Y. & Taylor, P.W. 2008. The β-lactam-resistance modifier (-)-epicatechin gallate alters architecture of the cell wall of Staphylococcus aureus. *Microbiology*, 153(7): 2093-2103. https://doi.org/10.1099/mic.0.2007/007807-0

Sun, L., Fan, X., Zeng, Y., Wang, L., Zhu, Z., Li, R., Tian, X., Wang, Y., Lin, Y., Wu, D. & Zeng, W. 2020. Resveratrol protects boar sperm in vitro via its antioxidant capacity. *Zygote*, 28(5): 417-424. https://doi.org/10.1017/S0967199420000271

Teixeira, S.M.P., Chaveiro, A. & Moreira Da Silva, F. 2015. The effects of three extenders on refrigerated boar semen. *South African Journal of Animal Science*, 45(1): 82-88. https://doi.org/10.4314/sajas.v45i1.10

Tvrdá, E., Bučko, O., Rojková, K., Ďuračka, M., Kunová, S., Kováč, J., Benko, F. & Kačániová, M. 2021. The Efficiency of Selected Extenders against Bacterial Contamination of Boar Semen in a Swine Facility in Western Slovakia. *Animals*, 11(11): 3320. https://doi.org/10.3390/ani11113320

Tvrdá, E., Straka, P., Galbavy, D. & Ivanic, P. 2019. Epicatechin Provides Antioxidant Protection to Bovine Spermatozoa Subjected to Induced Oxidative stress. *Molecules*, 24(18): 3226. https://doi.org/10.3390/molecules24183226

Tvrdá, E., Tušimová, E., Kováčik, A., Paál, D., Libová, Ľ. & Lukáč, N. 2016. Protective effects of quercetin on selected oxidative biomerkers in bovine spermatozoa subjected to ferrous ascorbate. *Reproduction in Domestic Animals*, 51: 524-537. https://doi.org/10.1111/rda.12714

Vestergaard, M. & Ingmer, H. 2019. Antibacterial and antifungal properties of resveratrol. *International Journal of Antimicrobial Agents*, 53(6): 716-723. https://doi.org/10.1016/j.ijantimicag.2019.02.015

Vongpralub, T., Thananurak, O., Sittikasamkit, C., Chuawongboon, P., Duangjinda, M., Boonkum, W. & Chankitisakul, V. 2016. Comparison of Effects of Different Antioxidants Supplemented to Long-term Extender on Boar semen Quality Following Storage at 17°C. *The Thai Journal of Veterinary Medicine*, 46(1): 119-126.

Waberski, D., Riesenbeck, A., Schulze, M., Weitze, K.F. & Johnson, L. 2019. Application of preserved boar semen for artificial insemination: Past, present and future challenges. *Theriogenology*, 137: 2-7. https://doi.org/10.1016/j.theriogenology.2019.05.030

Weber, D., Davies, M.J. & Grune, T. 2015. Determination of protein carbonyls in plasma cell extracts, tissue homogenates, isolated proteins: Focus on sample preparation and derivatization. *Redox Biology*, 5:367-380. https://doi.org/10.1016/j.redox.2015.06.005

Zhu, Z., Li, R., Fan, X., Lv, Y., Zheng, Y., Masudul Hoque, S.A., Wu, D. & Zeng, W. 2019. Resveratrol improves Boar Sperm Quality via 5'AMP-Activated Protein Kinase Activation during Cryopreservation. *Oxidative Medicine and Cellular Longevity*, 2019: 5921503. https://doi.org/10.1155/2019/5921503