



6th CASEE Conference
"Latest Trends in Bioeconomy in Danube Region"
May 24-26, 2015
Slovak University of Agriculture in Nitra, Slovak Republic

ANTIOXIDANT EFFICIENCY OF RESVERATROL ON OXIDATIVE STRESS-INDUCED DAMAGE IN BOVINE SPERMATOZOA

**Eva Tvrďá, Anton Kováčik, Eva Tušimová, Norbert Lukáč,
Jana Lukáčová, Peter Massányi**



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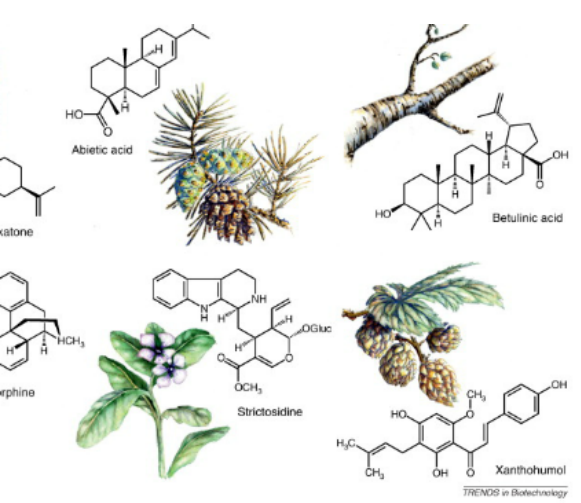
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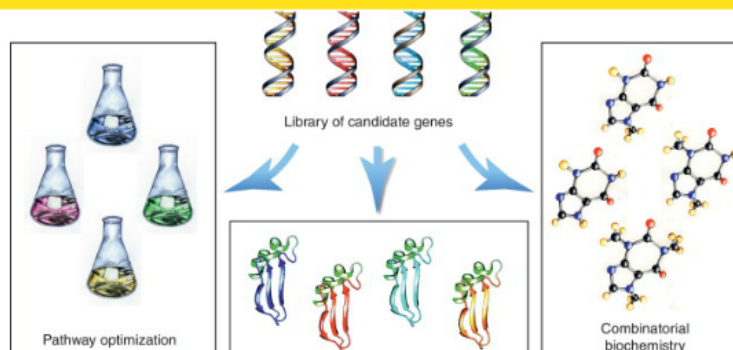


Natural Biologically Active Substances

Individual compounds extracted from vegetable raw materials reveal a wide range of biological activities used in healthcare, nutrition, intensive production and scientific research

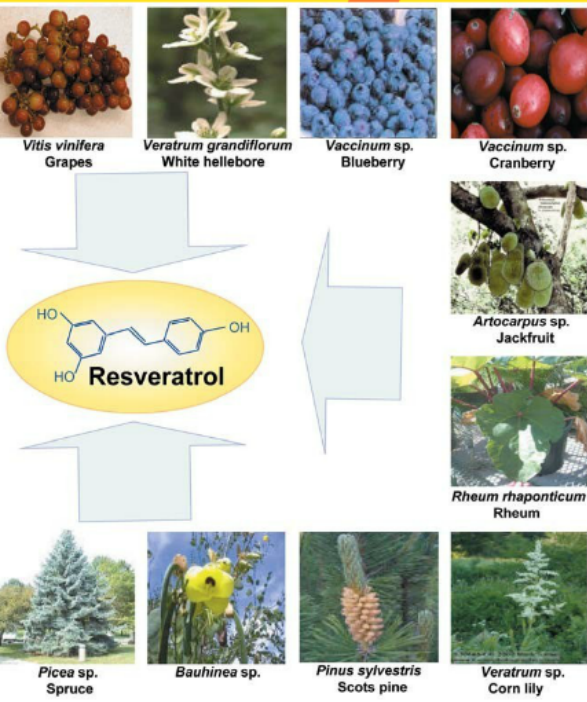
- Higher safety
- Potent pharmacological properties
- Chemical diversity, structural complexity, availability
- Lack of significant toxic effects, intrinsic biological activity

Structure	Effect on humans
	Narcotic pain killer
	Antimalarial drug
	Anticancer drug



Resveratrol

- (3,5,4'-trihydroxy-trans-stilbene) - a stilbenoid and a natural phenolic compound
- Phytoalexin produced naturally by several plants in response to injury, stress or when the plant is under attack by pathogens
- Food sources: skin of grapes, peanuts, cocoa, blueberries, raspberries, and mulberries

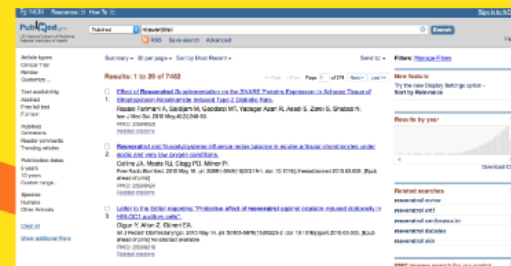
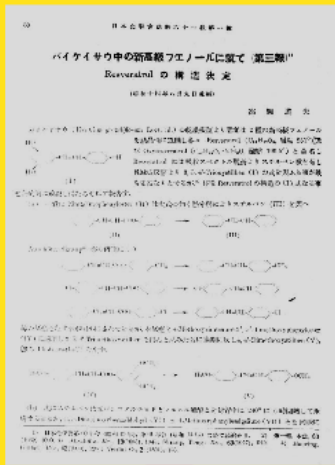
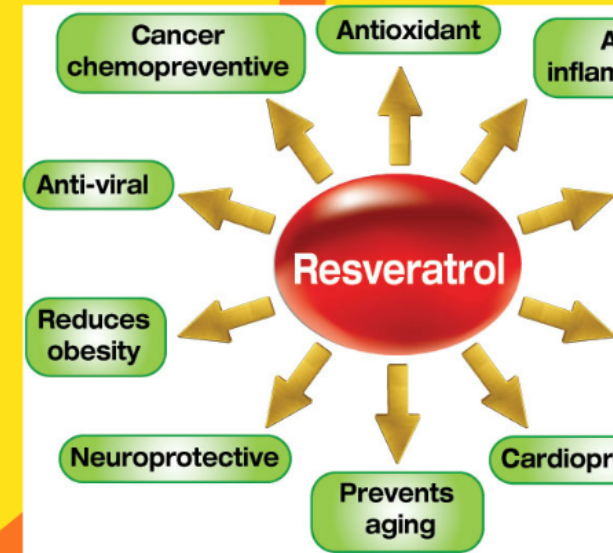
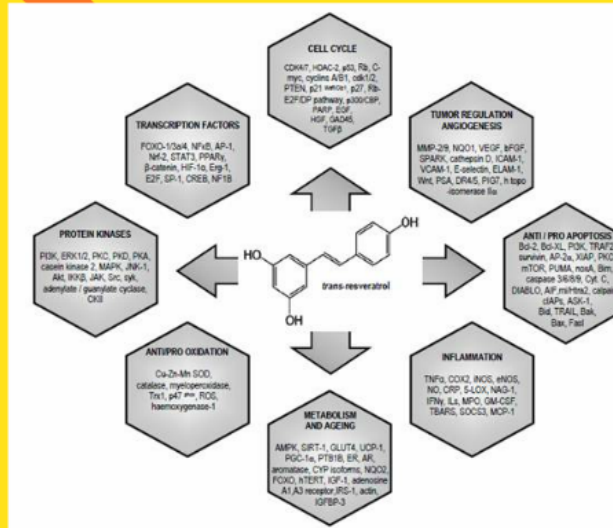


Product	Serving Size	Total Res
Red Wines	150 ml	650
Red Wines (Calif.)	150 ml	260
Roasted Peanuts	28 g	1
Peanut Butter	32 g	9.6
Cocoa Powder	10 g	18.3
Baking Chocolate	15 g	18.3
Dark Chocolate	40 g	14.1
Semisweet Baking Chips	15 g	7
Milk Chocolate	40 g	4
Chocolate Syrup	39 g	3



Health Effects of Resveratrol

- a Fountain of Youth?
- the French Paradox
- Possible health benefits
 - Slows the aging process
 - prevents cancer
 - prevents heart disease
 - improves cognitive function
 - Reduces appetite
 - Antioxidant properties



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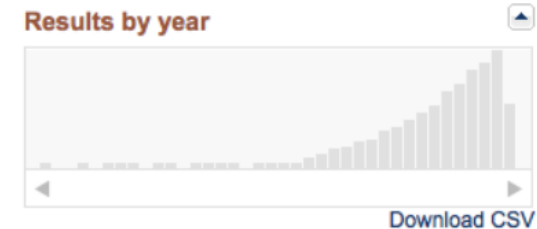
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Rezaei Farimani A, Saidijam M, Goodarzi MT, Yadegar Azari R, Asadi S, Zarei S, Shabab N. Iran J Med Sci. 2015 May;40(3):248-55.
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- [Resveratrol and N-acetylcysteine influence redox balance in equine articular chondrocytes under acidic and very low oxygen conditions.](#)
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- [Letter to the Editor regarding "Protective effect of resveratrol against cisplatin-induced ototoxicity in HEI-OC1 auditory cells".](#)
Olgun Y, Altun Z, Güneri EA. Int J Pediatr Otorhinolaryngol. 2015 May 14. pii: S0165-5876(15)00223-2. doi: 10.1016/j.ijporl.2015.05.003. [Epub ahead of print] No abstract available.
PMID: 25998215
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- [Determination of naturally occurring resveratrol analog trans-4,4'-dihydroxystilbene in rat plasma by liquid chromatography-tandem mass spectrometry: application to a pharmacokinetic study.](#)
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- [Stimulating basal mitochondrial respiration decreases doxorubicin apoptotic signaling in H9c2 cardiomyoblasts.](#)
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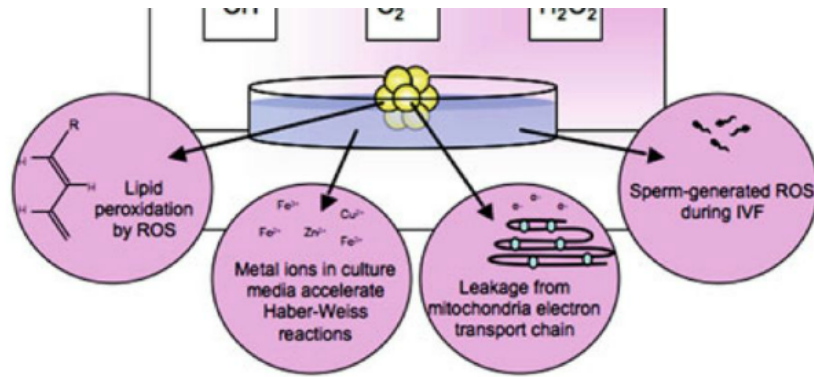
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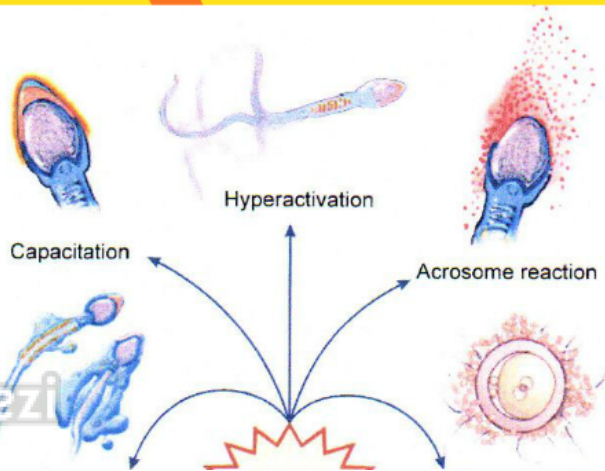




Oxidative Stress: a Key Factor in Subfertility

- Spermatozoa: particular sensitivity to oxidative stress development
- Diverse antioxidants may protect the spermatozoon against oxidative injury and subsequent dysfunction
- Essential for spermatozoa processing and culture routinely performed in andrology

Physiological roles of ROS
(Essential for sperm function)

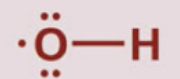


Oxidative Stress

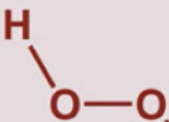
- | | | |
|-----------------------|-------------------|------------------------|
| Pentoxifylline | N-acetyl-cysteine | Folic acid |
| Selenium | Melatonin | Coenzyme Q10 |
| Vitamin E | Vitamin C | Glutathione peroxidase |
| Superoxide dismutase | EDTA | Glutathione |
| Nitric oxide synthase | Hypotaurine | Catalase |
| L-arginine | L-carnitine | Human serum albumin |



Superoxide anion



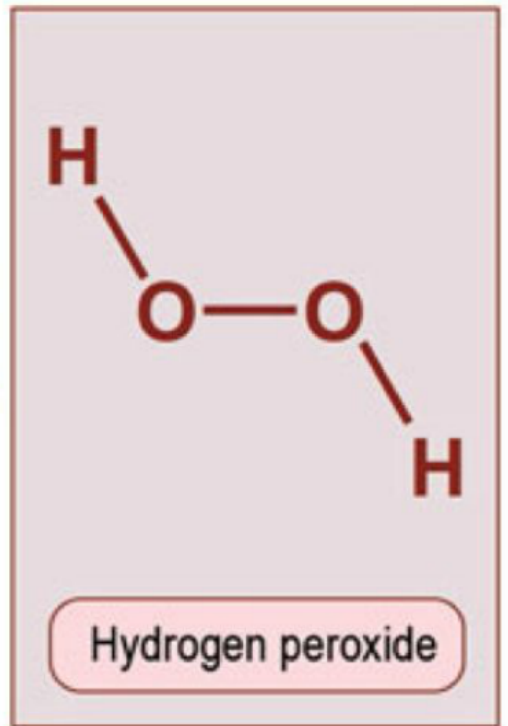
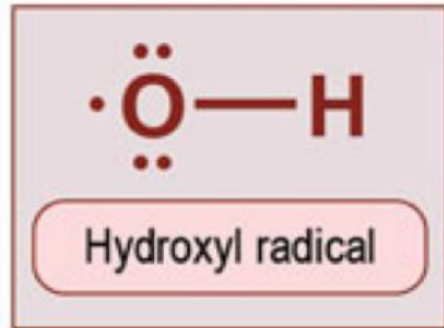
Hydroxyl radical



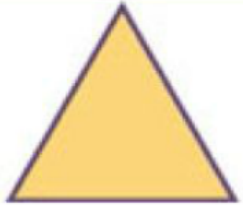
Hydrogen peroxide

Oxidative Stress

- Pentoxifylline
- N-acetyl-cysteine
- Folic acid
- Selenium
- Melatonin
- Coenzyme Q10
- Vitamin E
- Vitamin C
- Glutathione peroxidase
- Superoxide dismutase
- EDTA
- Glutathione
- Nitric oxide synthase
- Hypotaurine
- Catalase
- L-arginine
- L-carnitine
- Human serum albumin

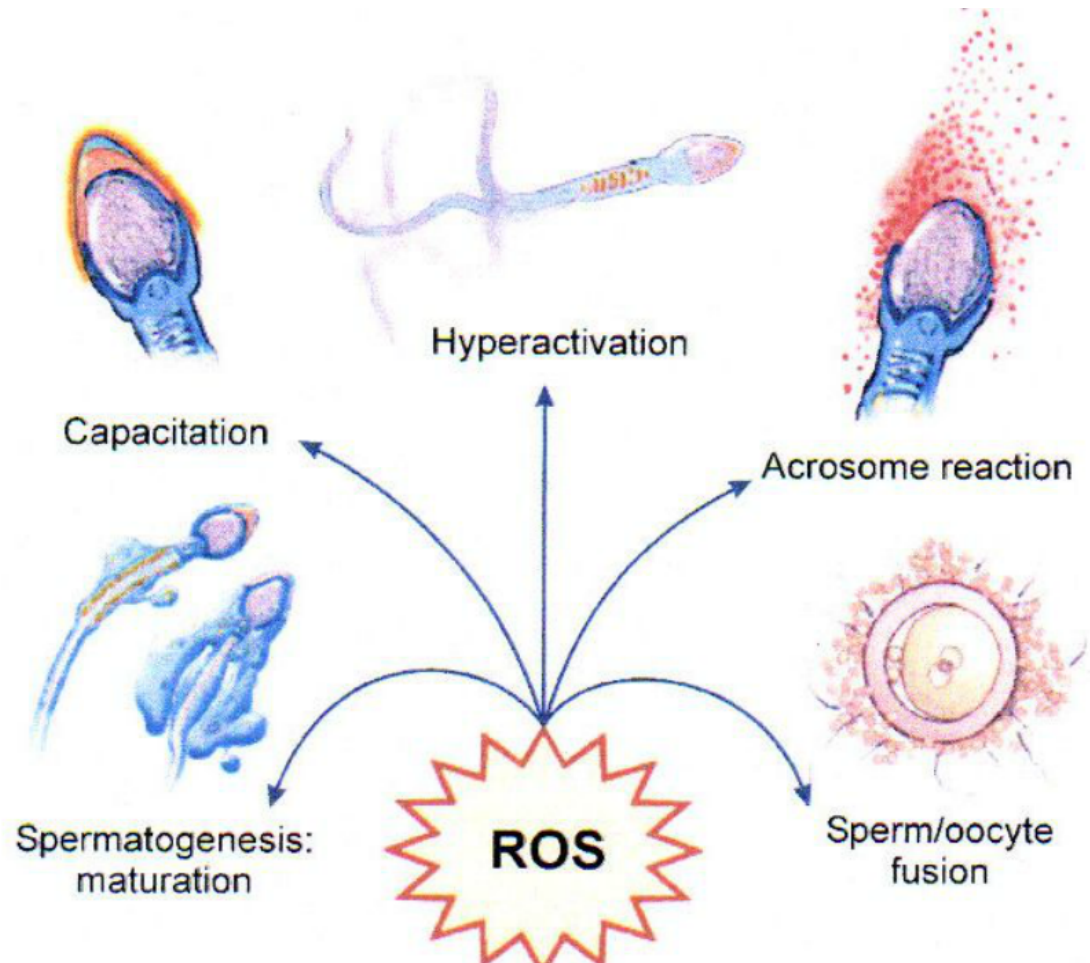


Antioxidants

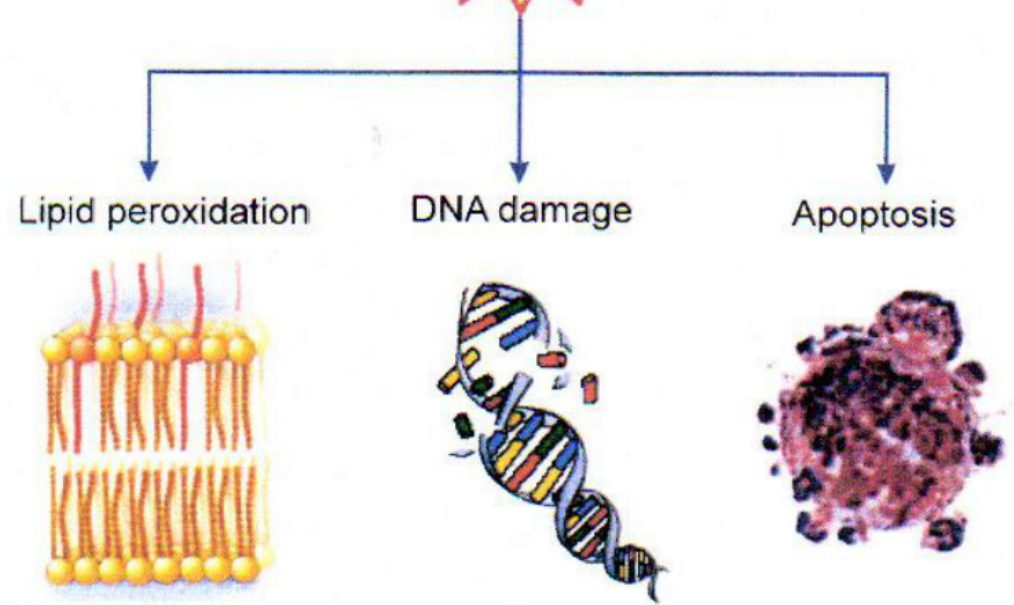


ROS

Physiological roles of ROS
(Essential for sperm function)



Pathological roles of ROS
(Lead to cell damage)



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present study was to develop a treatment supporting the men ejaculates collected from breeding rams was mixed and extended were completed with the following antioxidants: a ginseng (GP), Aromex® (AR), resveratrol (R), resveratrol + vitamin E (R+V), resveratrol + GP (RGP). Peroxidation was evaluated by the malondialdehyde (MDA) concentration in spermatozoa after incubation for 30, 60 and 120 min at 37°C as well as during a 24-hour incubation was checked in a 9-day-long period by observing the spermatozoa. Concentration of MDA was 4.06 nmol/10⁹ spermatozoa in control sample contained 69.79 nmol MDA per 10⁹ spermatozoa.

Mark Zuckerber... reverses cadmiu...

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The Journal of Reproduction

J Reprod Dev. 2014 Apr; 60(2): 115-127. Published online 2014 Feb 1. doi: 10.1262/jrd.2013.0115

Resveratrol Reverses Cadmium-Induced Infertility by Downregulating Bcl-2 Gene Expression

Samy M. ELEAWA,¹ Mahmoud A. ALKHATEIB,¹ Hesham M. ELREFAEY,³ Abbas O. ELKARIE,⁴ Shatour,⁵ and Mohammad A. KHAILIL⁵

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Abstract

This study was performed to investigate the effect of resveratrol on the fertility of male rats. Sixty rats were divided into six groups: A) controls+NS, B) control+vehicle, C) CdCl₂+NS, D) CdCl₂+vehicle, E) CdCl₂+vehicle+resveratrol, and F) CdCl₂+vehicle+resveratrol+NS. At the end of the protocol, serum levels of

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Original Article

Resveratrol appears to protect against oxidative stress and steroidogenesis collapse in mice fed high-calorie and high-cholesterol diet

H.-J. Wang^{1,†}, Q. Wang^{1,†}, Z.-M. Lv^{1,†}, C.-L. Wang², C.-P. Li² and Y.-L. Rong²

Article first published online: 23 JAN 2014
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Keywords:

Leydig cell; obesity; oxidative stress; resveratrol; steroidogenesis

Summary

The detrimental effects on Leydig cells steroidogenesis in mice on high-calorie and high-cholesterol diet (HCD) were determined, and the

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Aims of the Study

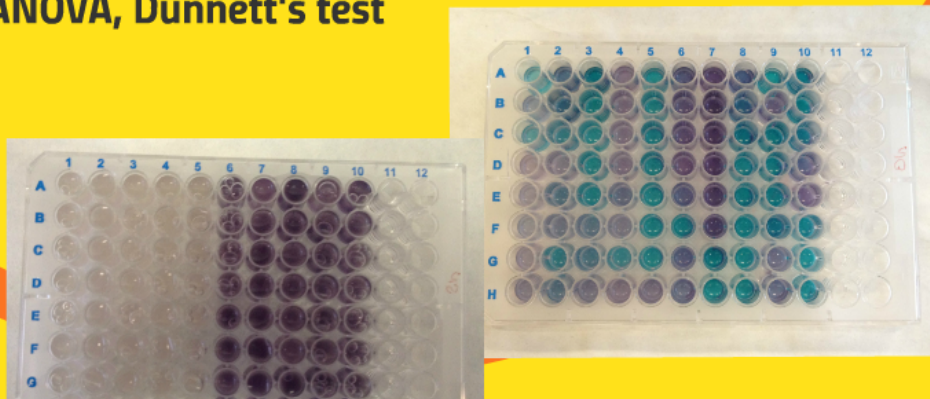
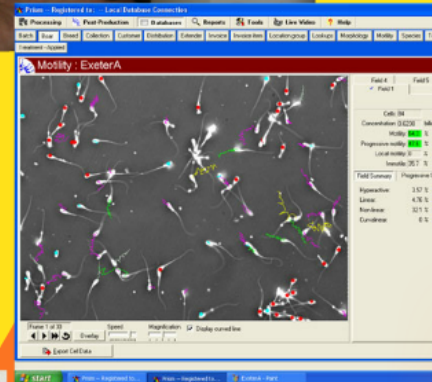
To evaluate the *in vitro* antioxidant activity of resveratrol against oxidative stress induced by ferrous ascorbate in bovine spermatozoa





Material and Methods

- Semen samples from 25 breeding bulls
- Sperm isolation and re-suspension in 2.9% sodium citrate
- *In vitro* culture (37 °C, 6h)
- Sperm motility analysis: Computer-aided sperm analysis (CASA)
- Superoxide production: Nitroblue-Tetrazolium (NBT) Test
- Cell lysis
- Colorimetric/spectrophotometric analysis of:
 - Superoxide dismutase activity
 - Catalase activity
 - Glutathione availability
 - Lipid peroxidation/Malondialdehyde production
- Statistical analysis: Descriptive statistical characteristics, One-way ANOVA, Dunnett's test

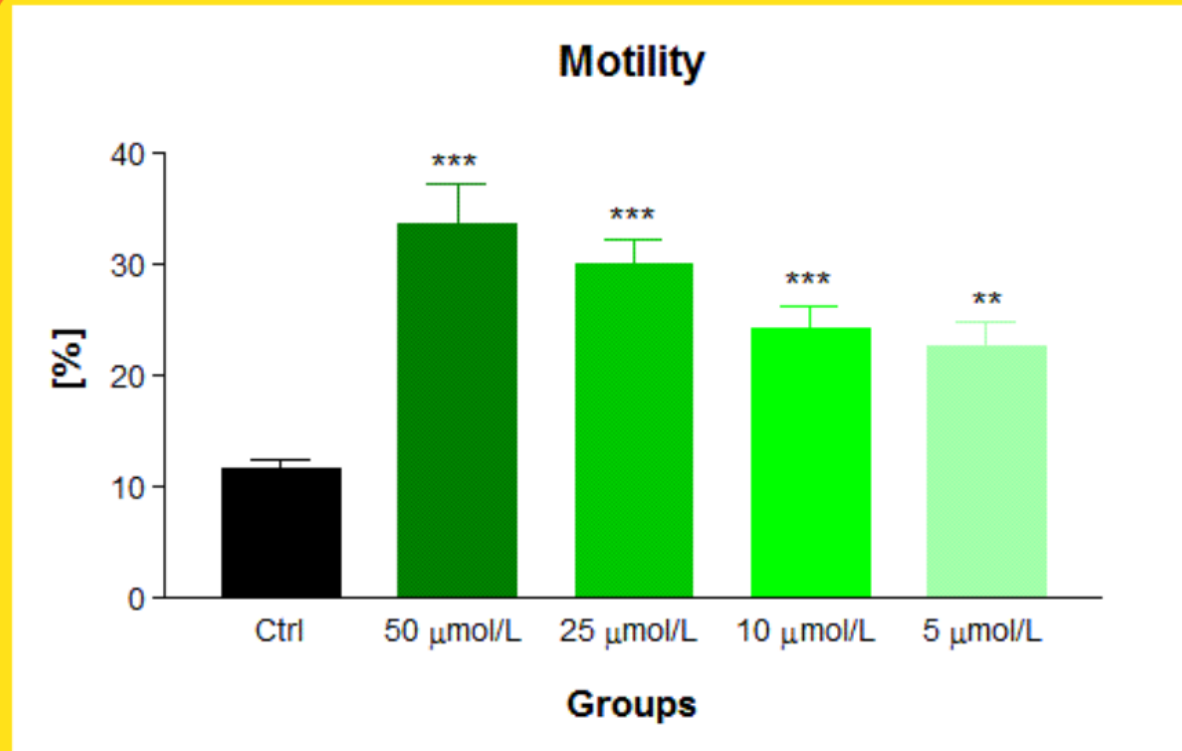


Experimental Groups

GROUP	TREATMENT
Ctrl	Ferrous Ascorbate (FeAA) 150 $\mu\text{mol/L}$ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ + 750 $\mu\text{mol/L}$ ascorbic acid
A	FeAA + 50 $\mu\text{mol/L}$ RES
B	FeAA + 25 $\mu\text{mol/L}$ RES
C	FeAA + 10 $\mu\text{mol/L}$ RES
D	FeAA + 5 $\mu\text{mol/L}$ RES

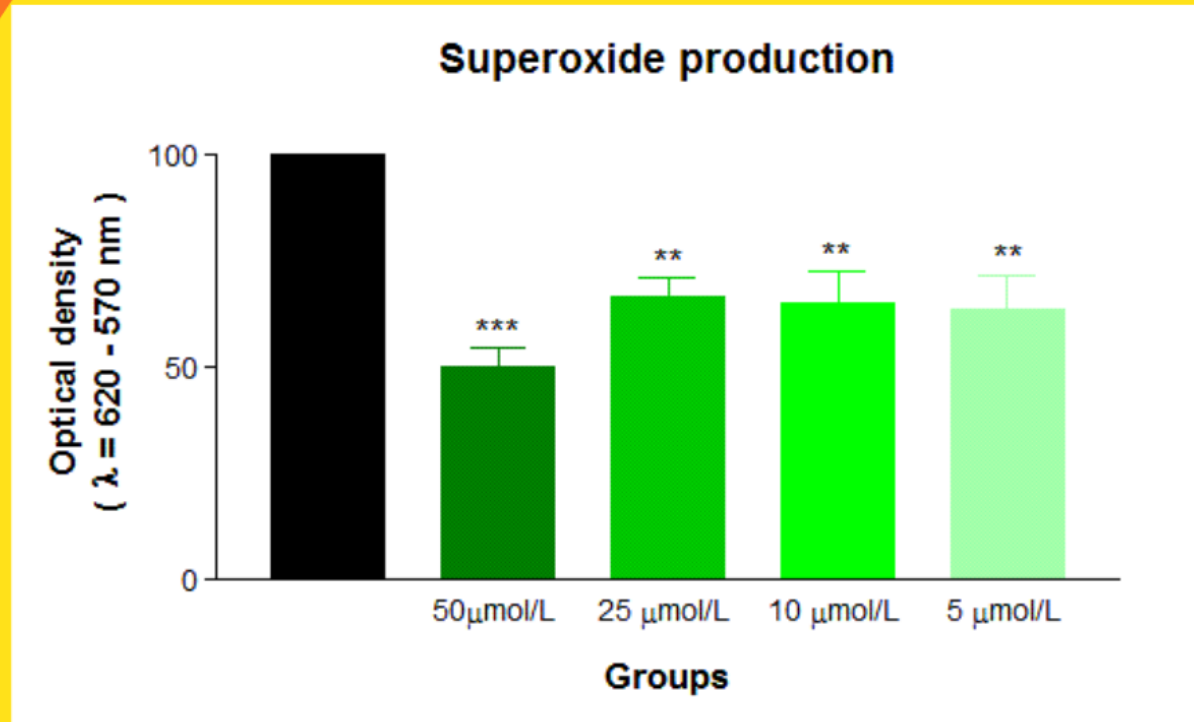


Results: Spermatozoa Motility

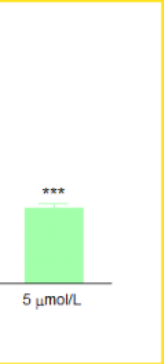


*** ($P < 0.001$); ** ($P < 0.01$); * ($P < 0.05$)

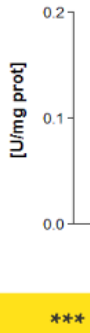
Results: NBT Test



*** ($P < 0.001$); ** ($P < 0.01$); * ($P < 0.05$)



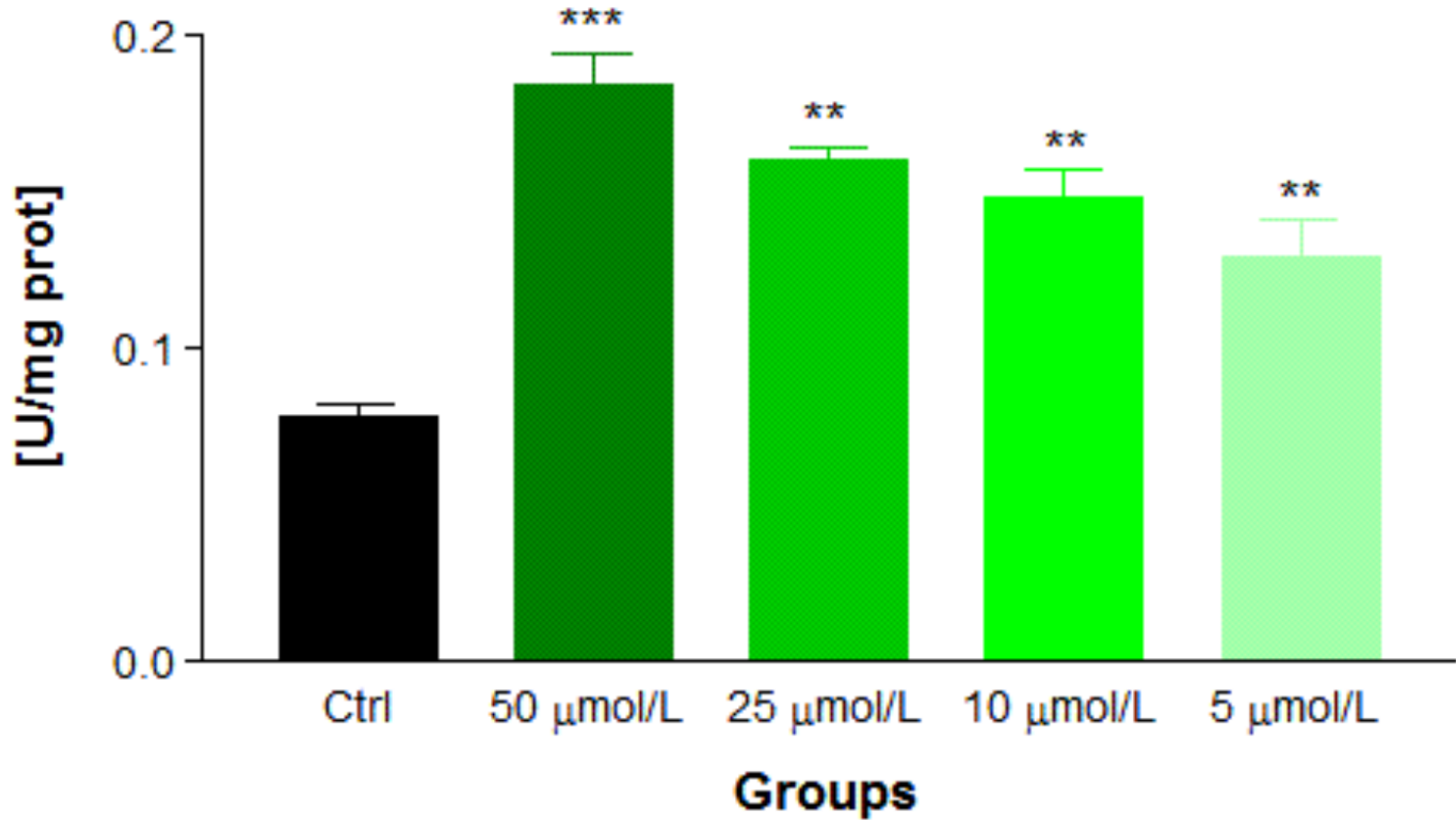
P<0.05)



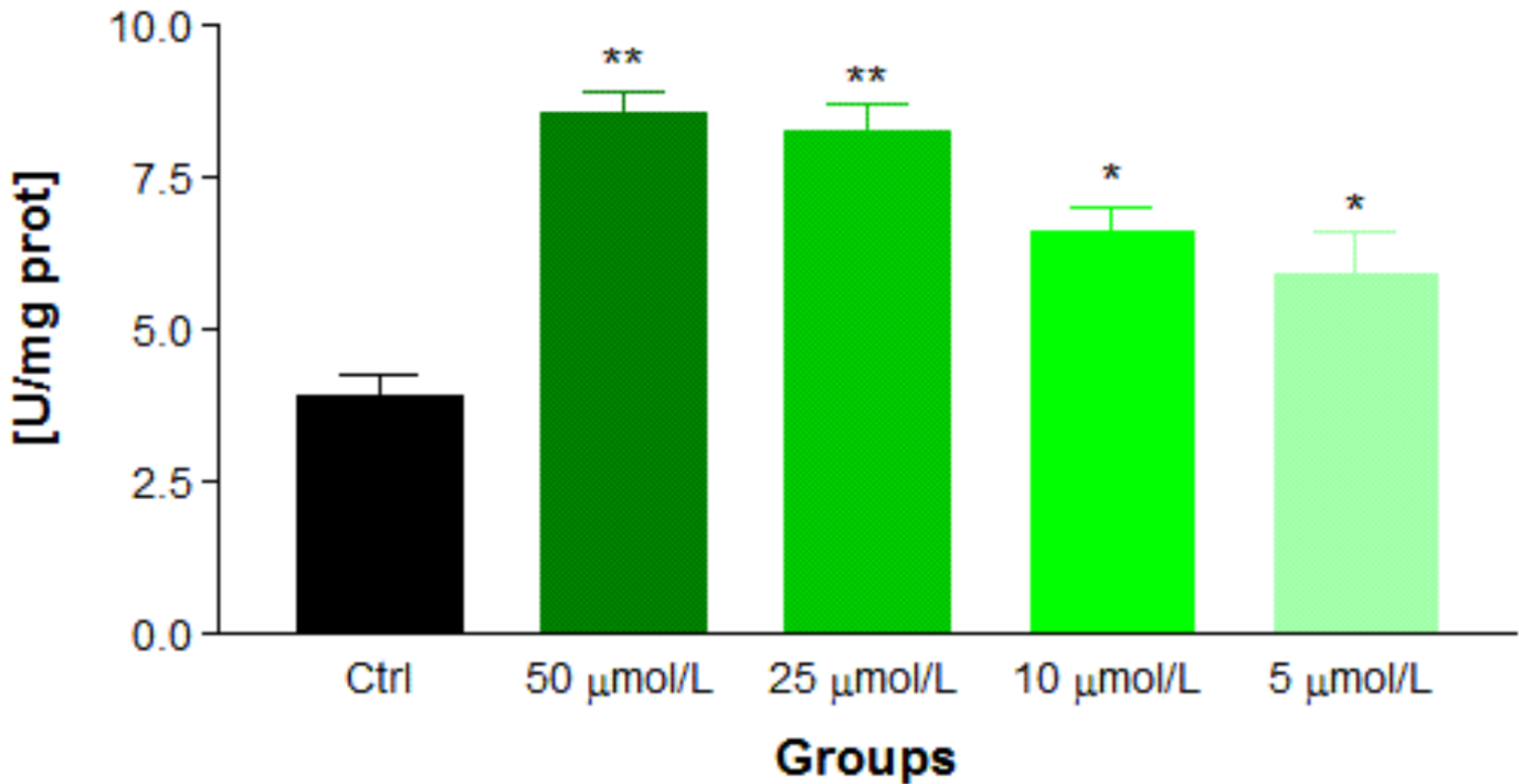
Results: Oxidative Profile

- **Superoxide Dismutase Activity**
- **Catalase Activity**
- **Glutathione Availability**
- **Malondialdehyde Production**

Superoxide Dismutase



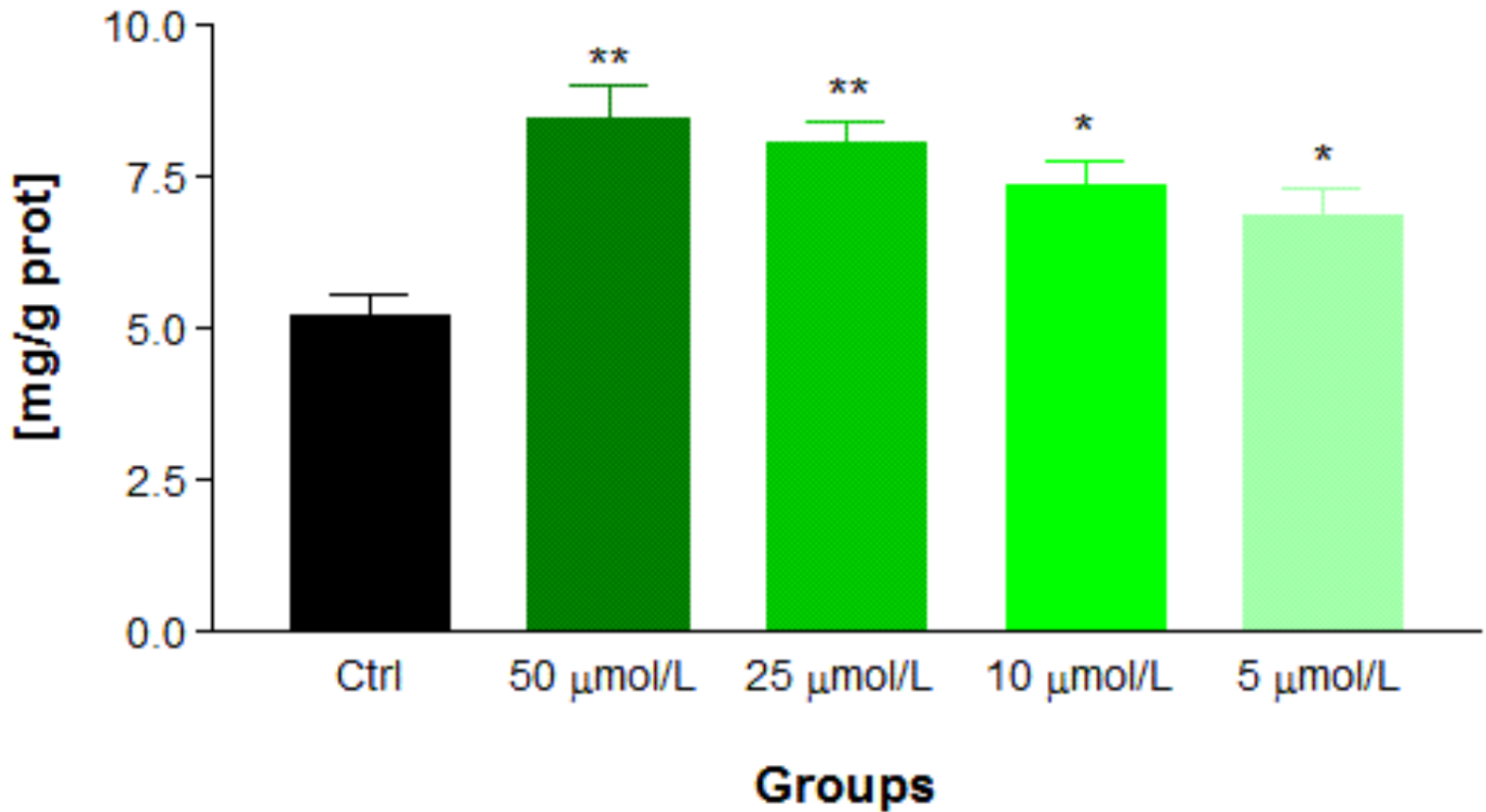
Catalase



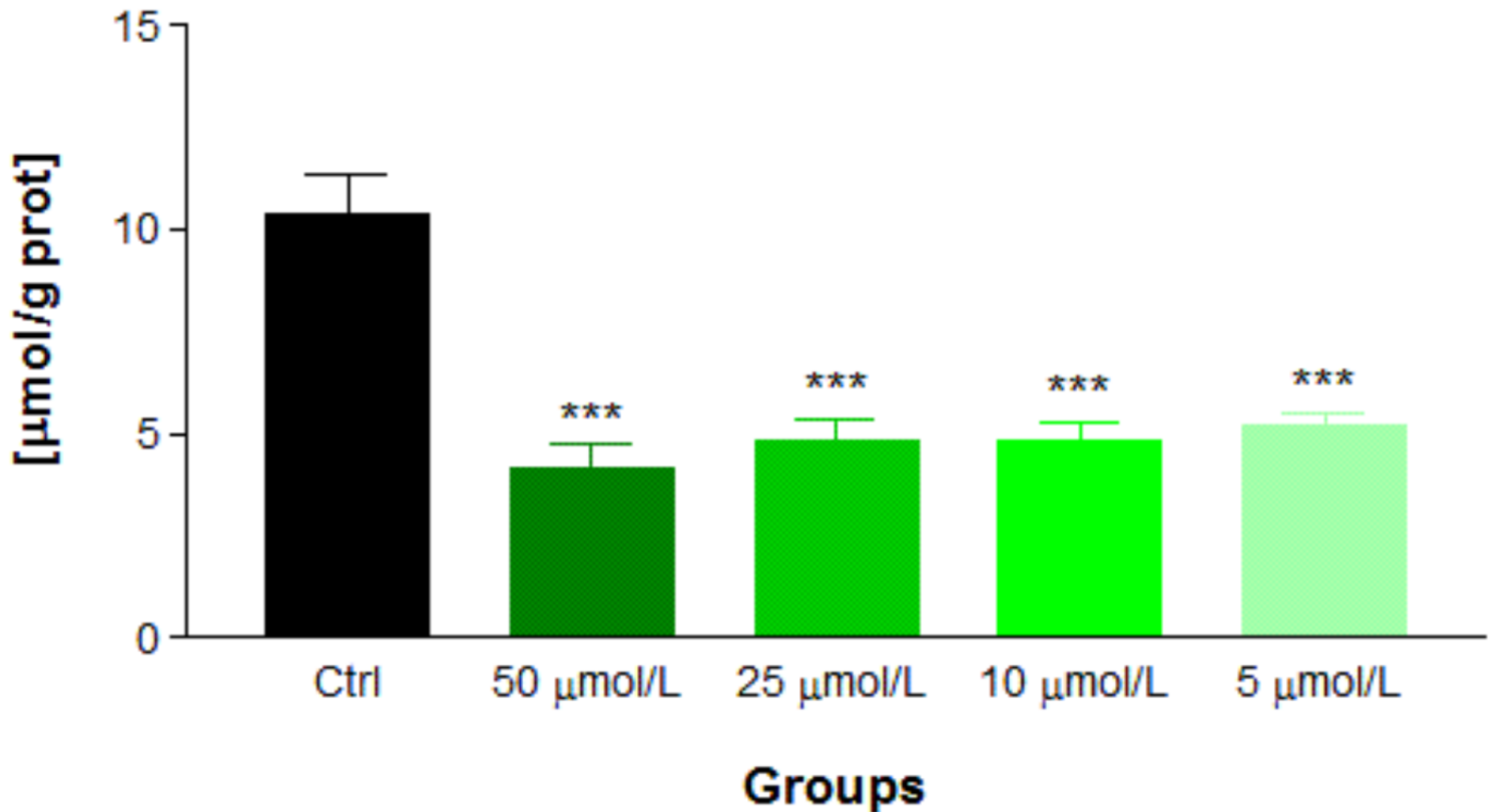
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*** (P<0.001); ** (P<0.01); * (P<0.05)

Glutathione



Malondialdehyde

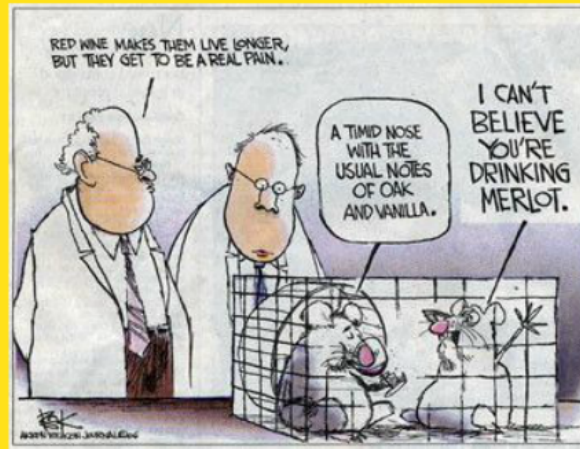


Conclusions

- Resveratrol was able to prevent the decline of spermatozoa vitality and antioxidant capacity as a consequence of FeAA-associated oxidative stress development
- Most effective resveratrol concentrations: 25–50 $\mu\text{mol/L}$
- Notable mechanisms of action: protection against lipid peroxidation and stabilization of enzymatic antioxidants
- Resveratrol administration may be a suitable strategy to prevent damage to the sperm structural integrity and functional activity

TAKE HOME MESSAGE

- A combination of iron and ascorbic acid may have deleterious effects on sperm vitality and function
- Resveratrol offers antioxidant stimulation and protection to spermatozoa under oxidative stress conditions
- Resveratrol may have modulating effects on antioxidant enzymes
- Spermatozoon may autoregulate its antioxidant defense mechanisms in response to changing environmental conditions



THANK YOU FOR YOUR ATTENTION

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