BENEFICIAL EFFECTS OF ISOQUERCITRIN ON THE BEHAVIOR OF BOVINE SPERMATOZOA *IN VITRO*

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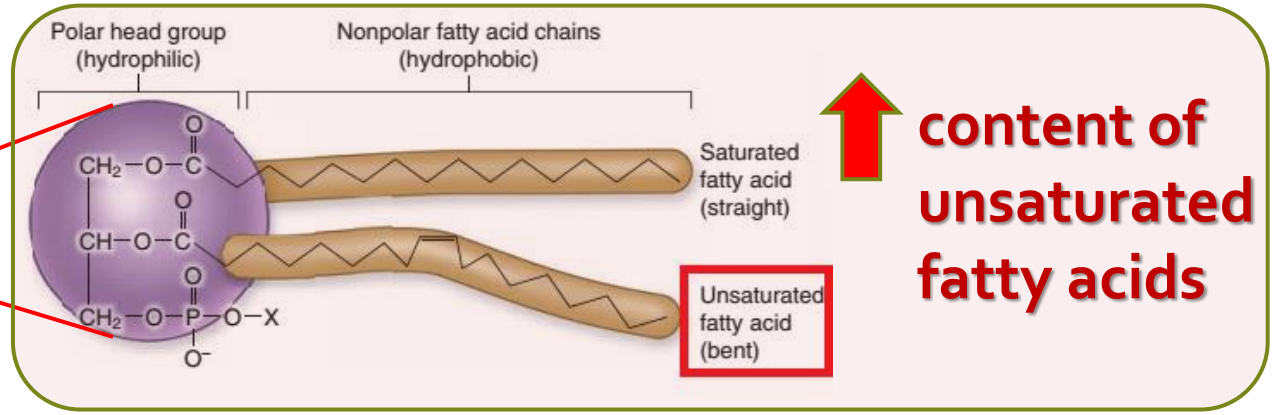
"Sustainable development in Europe – cooperation between science and practice - What's the position of Central and South Eastern Europe?"

Introduction

Oxidative stress and sperm

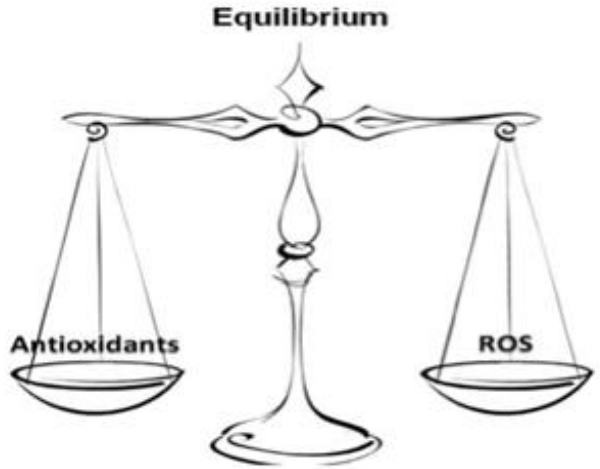
- Spermatozoa are especially sensitive to peroxidative damage

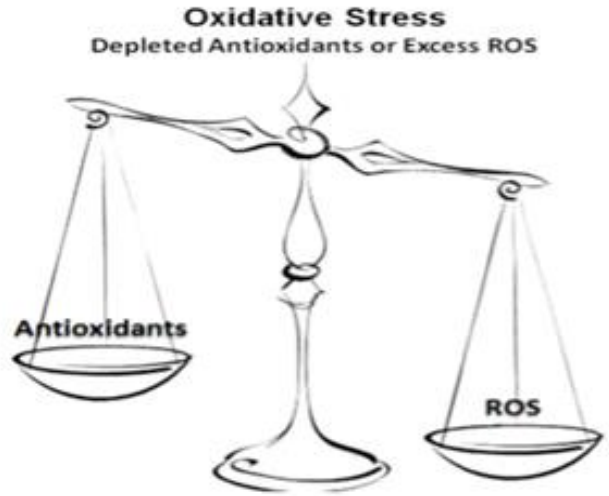


content of unsaturated fatty acids

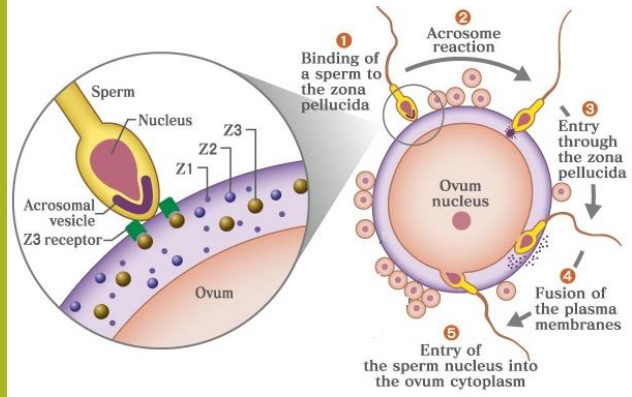
antioxidant capacity

Oxidative stress and sperm



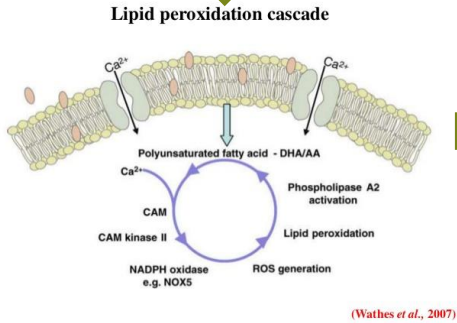


- **Low ROS levels provide optimal conditions for several main sperm functions:**



- capacitation
- acrosome reaction
- *zona pellucida* binding
- oocyte fusion

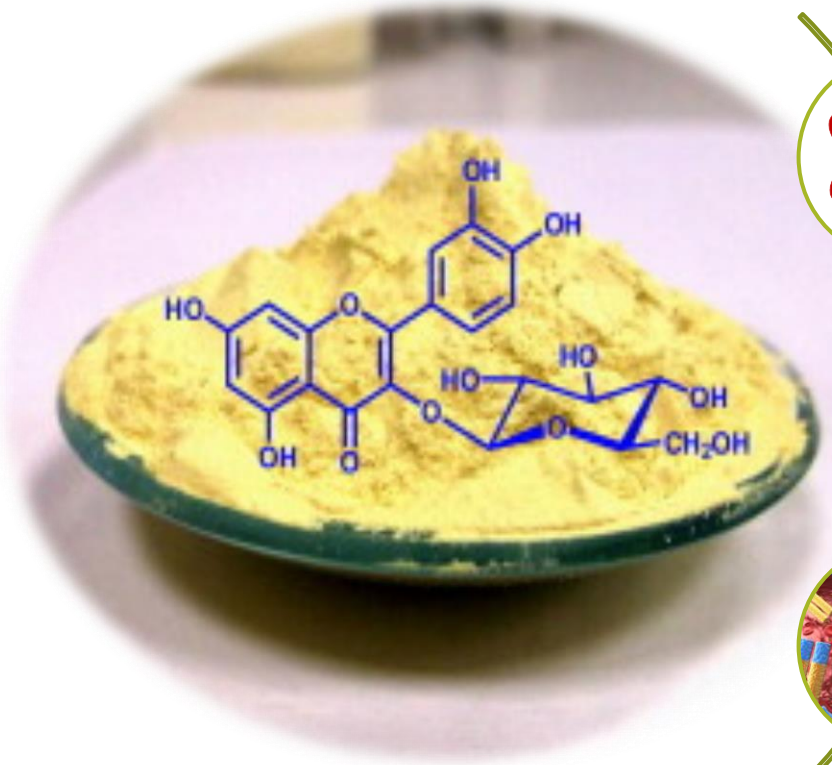
- **Overproduction of ROS and imbalance between ROS and antioxidant capacity of cells leads to OXIDATIVE STRESS**



reduced motility and viability → reduced fertilizing ability

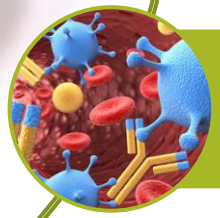
Isoquercitrin

- Isoquercitrin (quercetin-3-*O*-β-D-glucopyranoside) is, together with rutin, one of the major glycosidic forms of the natural flavonol quercetin
- Higher bioavailability than quercetin



Antioxidant effect

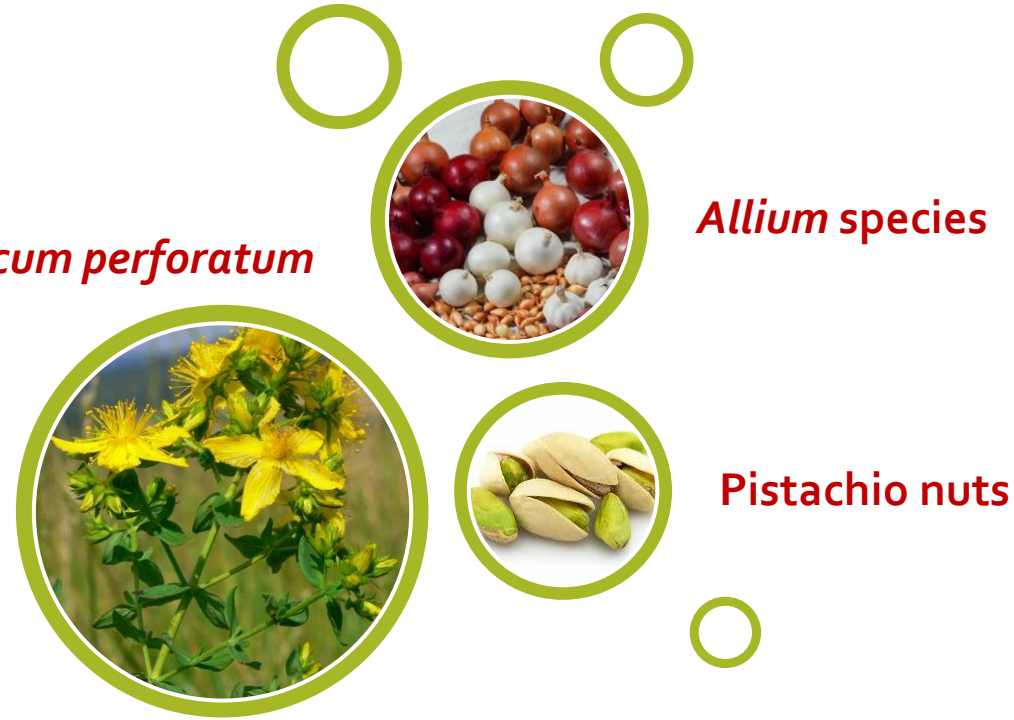
Anticarcinogenic effect



Anti-inflammation effect

Isoquercitrin

- Isoquercitrin is commonly found in medicinal herbs, fruits, vegetables and plant-derived foods and beverages

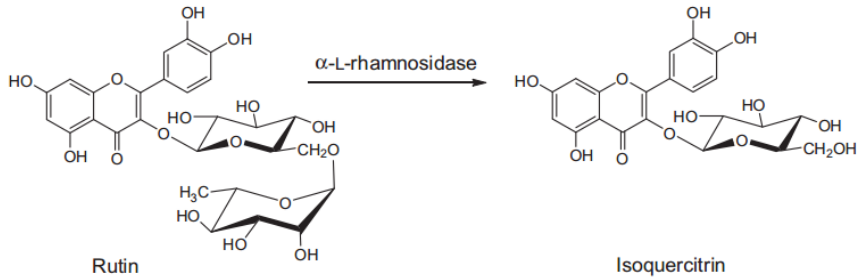


Hypericum perforatum

Allium species

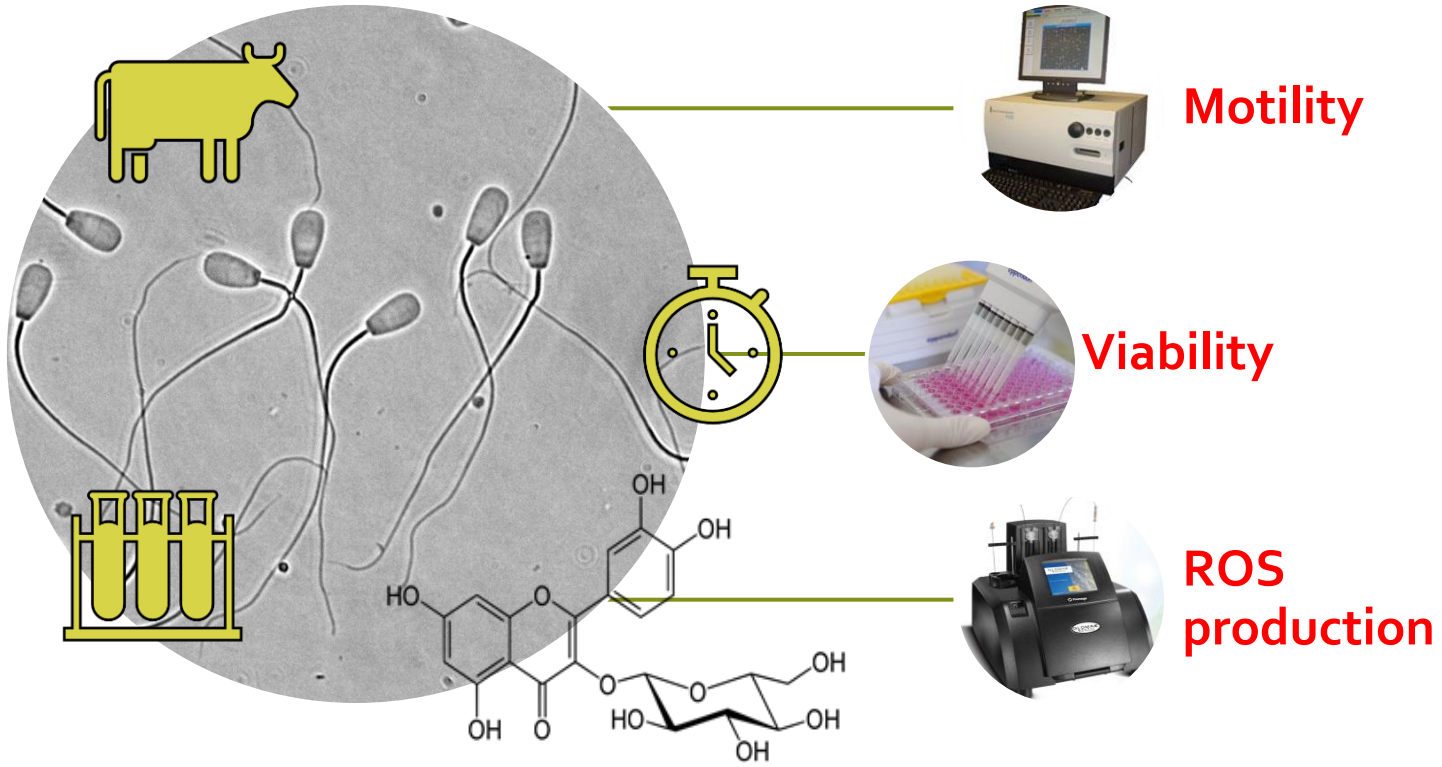
Pistachio nuts

- Pure isoquercitrin can be obtained on a large scale by enzymatic rutin hydrolysis with a-L-rhamnosidase



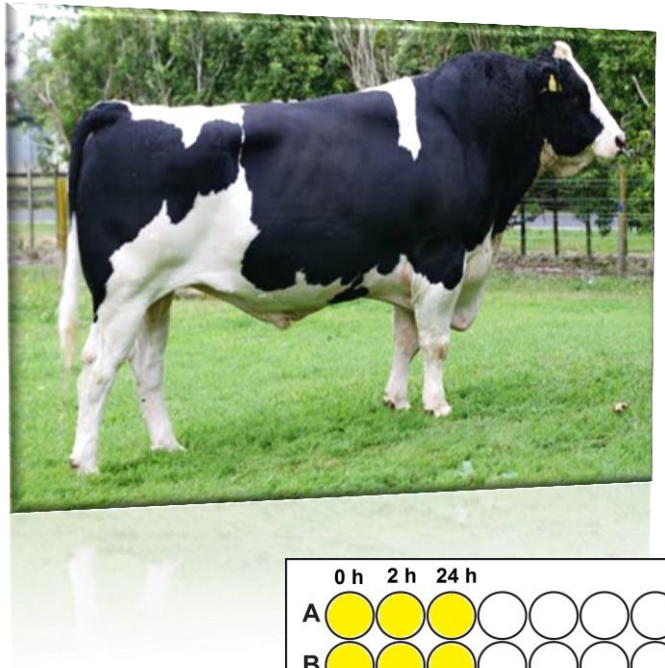
Aim of study

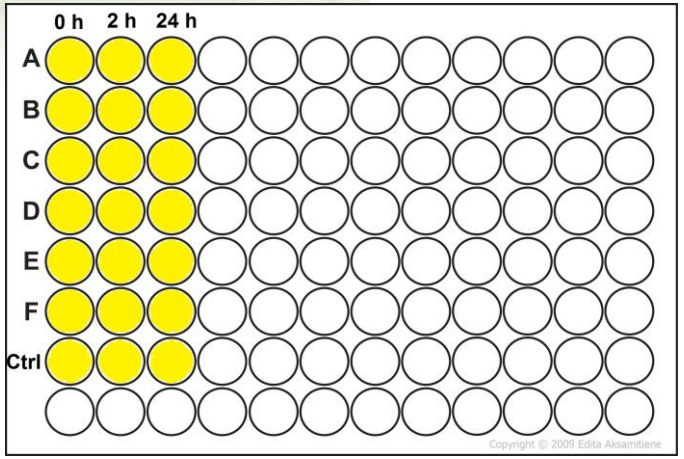
- Evaluate the dose- and time-dependent *in vitro* antioxidant effect of isoquercitrin on bovine spermatozoa during three different time periods (0 h, 2 h, 24 h)



Material and methods
Semen samples and *in vitro* culture

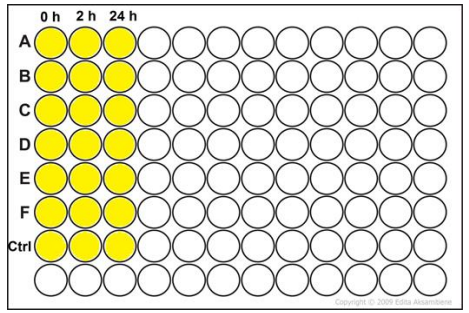
- Bovine semen samples were obtained from adult breeding bulls
- Each sample was diluted in physiological saline solution, using a dilution ratio of 1:40
- Spermatozoa were incubated with various concentrations (A – 200; B – 100; C – 50; D – 10; E – 5; F – 1 µmol.l^{-1}) of isoquercitrin dissolved in 0.5% DMSO
- Results from all analyzes were collected during three repeated experiments at each concentration

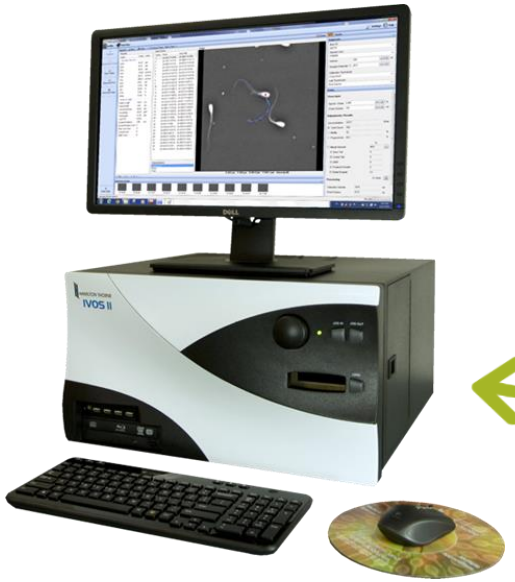




Material and methods
Computer-assisted semen analysis

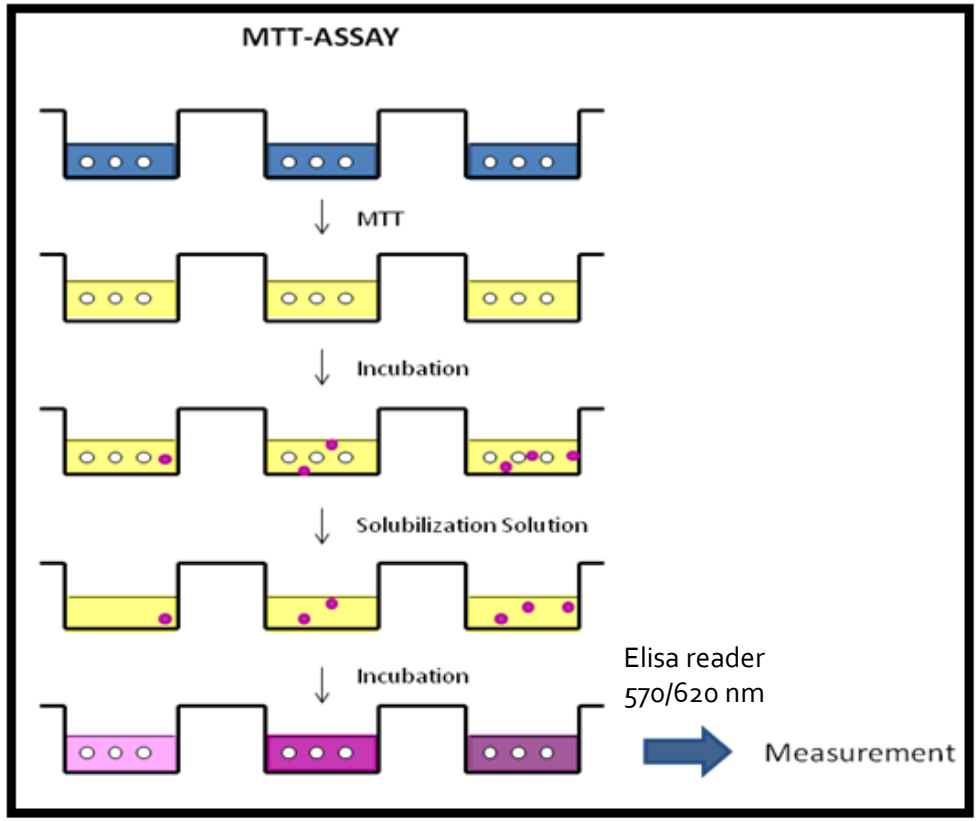
- The motility analysis was carried out using a CASA (Computer Assisted Semen Analyzer) system – HTM IVOS (CASA; Version 14.0 TOX IVOS II.; Hamilton-Thorne)
- Each sample was placed into the Makler Counting Chamber and the percentage of motile spermatozoa (motility > 5 µm/s) was evaluated
- At least 1000 spermatozoa were analyzed in each sample

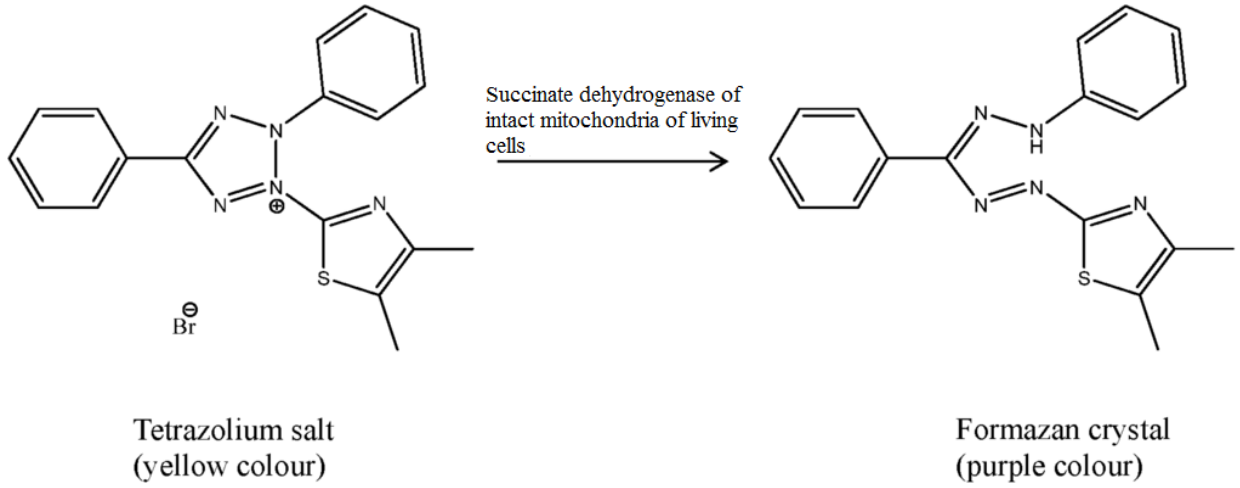




Material and methods
Viability evaluation

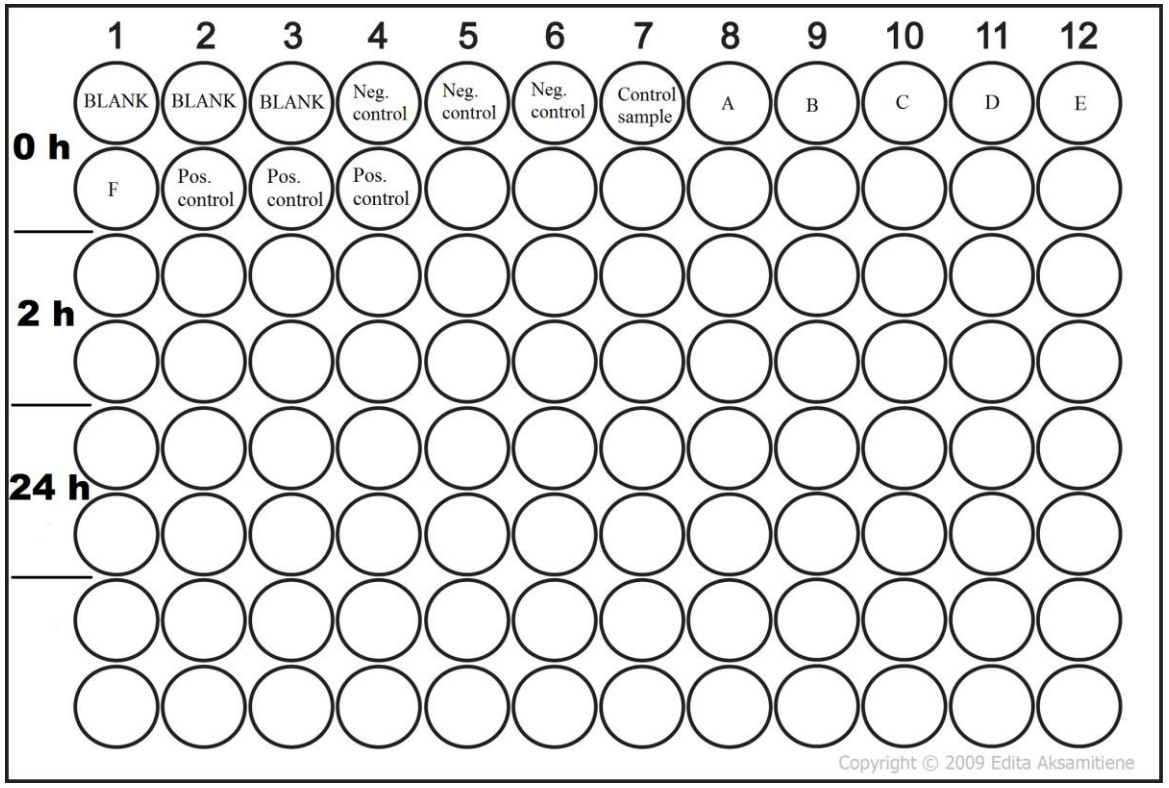
- The viability of the sperm cells was evaluated by the metabolic activity (MTT) assay





Material and methods
Evaluation of ROS generation

- ROS levels were measured by chemiluminescence assay using luminol (5-amino- 2, 3-dihydro-1, 4-phthalazinedione)

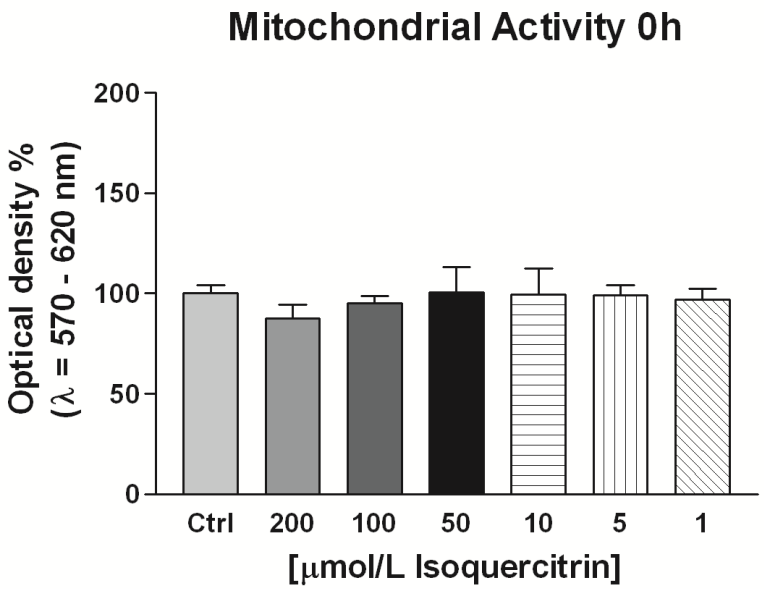


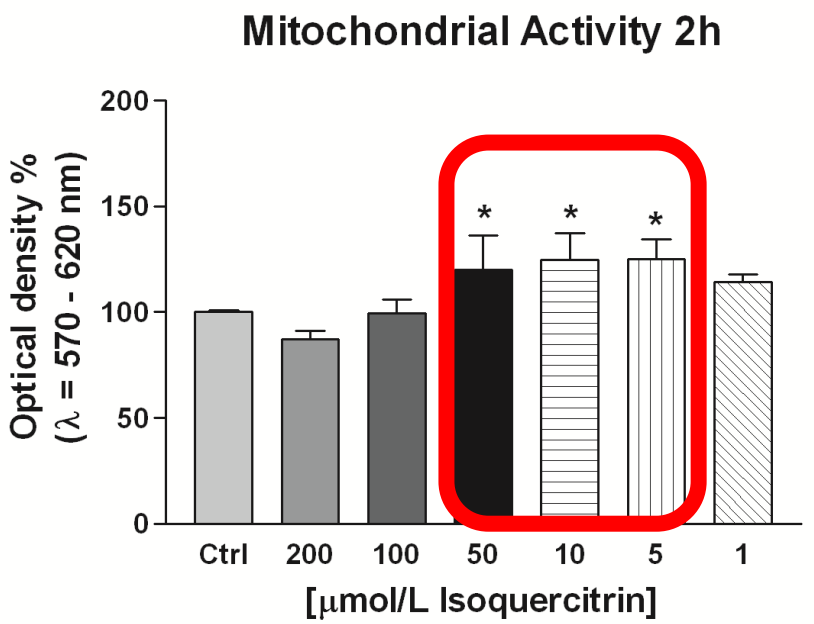
Results
Motility (%)

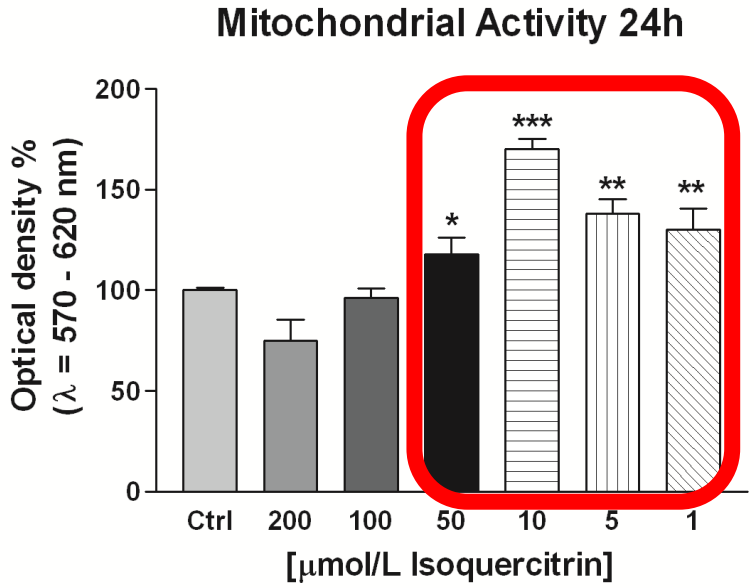
Groups/ Time	Ctrl	A	B	C	D	E	F
0h	75.67±3.79	65.33±1.98	66.67±1.90	80.33±3.12	79.33±4.79	77.33±2.73	79.33±3.60
2h	61.67±1.54	50.67±6.38	59.67±5.66	64.33±1.54	73.33±2.01	69.33±2.92	62.33±4.51
24h	1.33±0.62	11.33±3.68	14.00±2.55	17.33±3.70*	19.33±1.12*	17.00±2.12*	15.33±1.73

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

Results
Viability evaluation (%)







Each bar represents mean (±SEM) optical density as the percentage of controls, which symbolize 100%.
*** P<0.001; ** P<0.01; *P<0.05

Results
Level of ROS (RLU/sec/10^6 sperm)

Groups/ Time	Ctrl	A	B	C	D	E	F
0h	2.02±0.23	1.60±0.16	1.47±0.20	1.42±0.10	1.20±0.11	1.29±0.12	1.53±0.25
2h	3.60±0.26	4.36±0.20	3.86±0.15	3.09±0.21	2.66±0.19	3.05±0.24	3.56±0.22
24h	15.46±0.70	16.17±0.87	15.55±0.99	11.49±0.69***	10.92±0.75***	11.44±0.90***	15.27±0.82

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

Conclusion

The present study demonstrated improved viability, motility and also decline of ROS production after short- and long-term storage of bovine spermatozoa with administration of isoquercitrin.

Isoquercitrin is able scavenge reactive oxygen species, particularly after administration of isoquercitrin in concentrations ranging between 5 and 50 $\mu mol.l^{-1}$, with more significant differences after long-term cultivation (24 h).

These results indicate, that isoquercitrin may be able to enhance fertilizing ability of sperm storage for further processing.

THANK YOU FOR YOUR ATTENTION

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