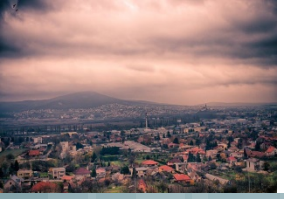

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Department of Animal Physiology

LYCOPENE OFFERS PROTECTION AGAINST OXIDATIVE DAMAGE IN FROZEN-THAWN BOVINE SEMEN

7th CASEE Conference
"The Role of Life Sciences in Europe's 2020 Strategy"
May 22 - 24, 2016
Timisoara, Romania

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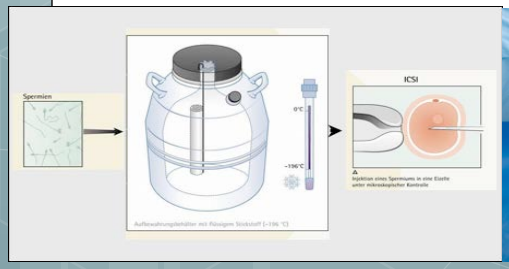
Sperm Cryopreservation

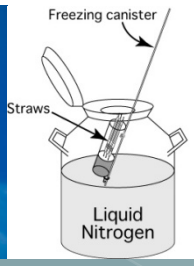
Benefits:

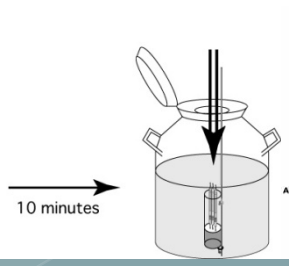
- Improvement of genetic quality and health condition of the animals
- Significant cost reduction for the production maintenance
- A valuable tool for assisted reproduction techniques in veterinary andrology
- An important technique for the protection of genetic potential and biodiversity endangered species
- Economic preservation of transgenic animal lines

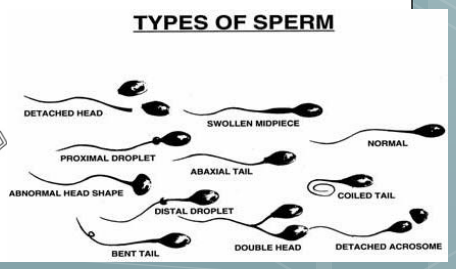
Issues:

- Substantial loss of cellular mass (50-60%)
- Mechanical damage and thermal stress
- Oxidative stress

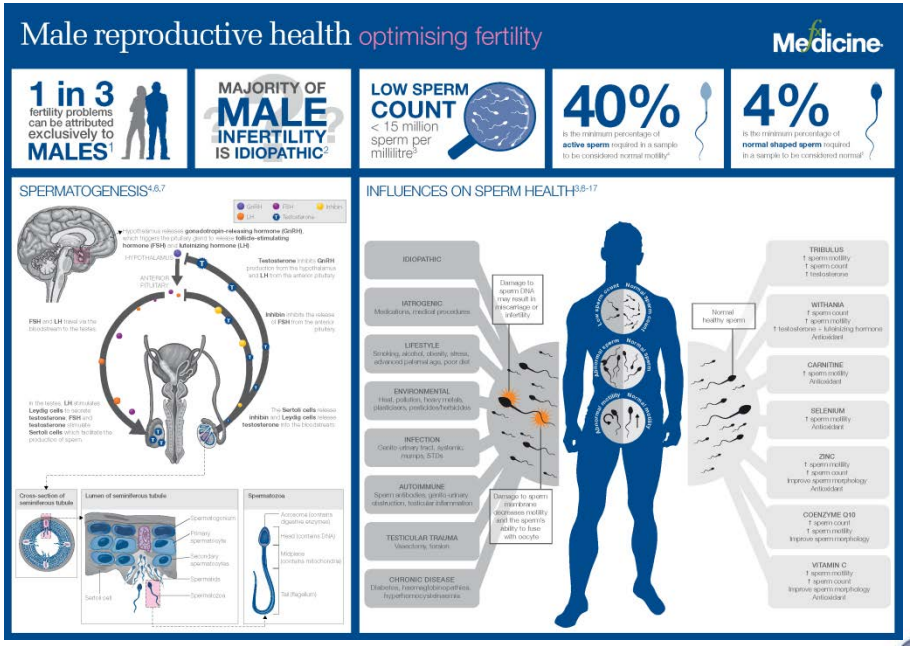


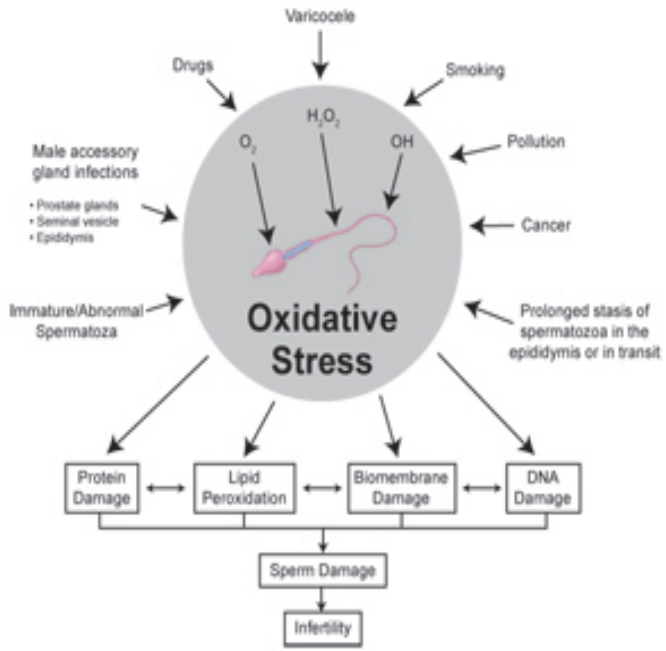






Oxidative Stress



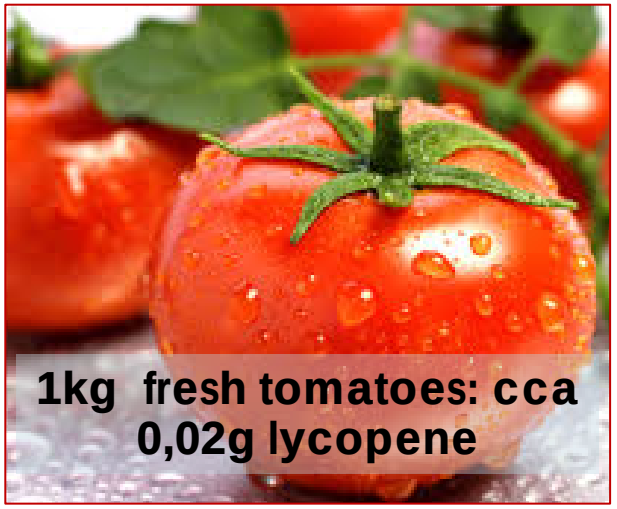


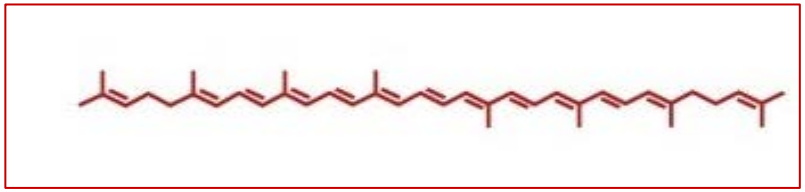
Sperm have a special membrane that allow them to fuse with an egg during fertilisation.

Oxidative stress can **damage this membrane** preventing fertilisation.

Lycopene (LYC)

- The most abundant carotenoid found in nature, and a highly efficient antioxidant
- Proposed to be involved in processes related to the quenching of singlet oxygen and trapping of peroxy radicals
- Previous studies: LYC has the ability to improve sperm motility, membrane integrity and to protect DNA damage in spermatozoa





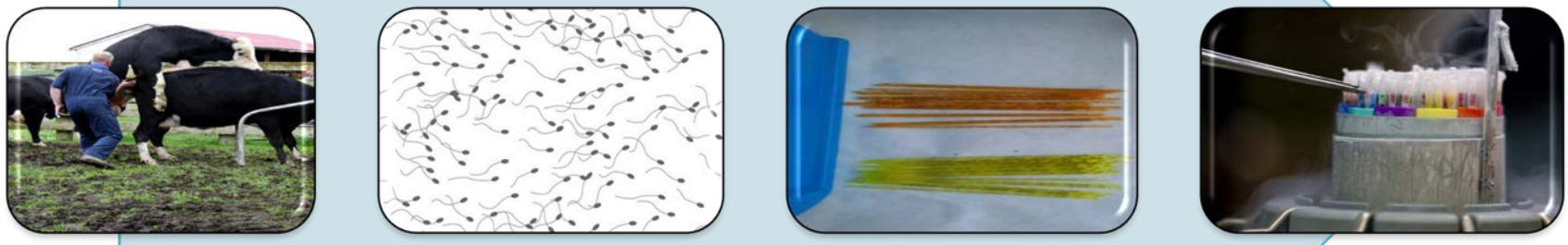
Other sources:

AIM OF THE STUDY

- To evaluate the effects of lycopene on:
  - sperm motility
  - reactive oxygen species (ROS) production
  - lipid peroxidation in cryopreserved bovine semen

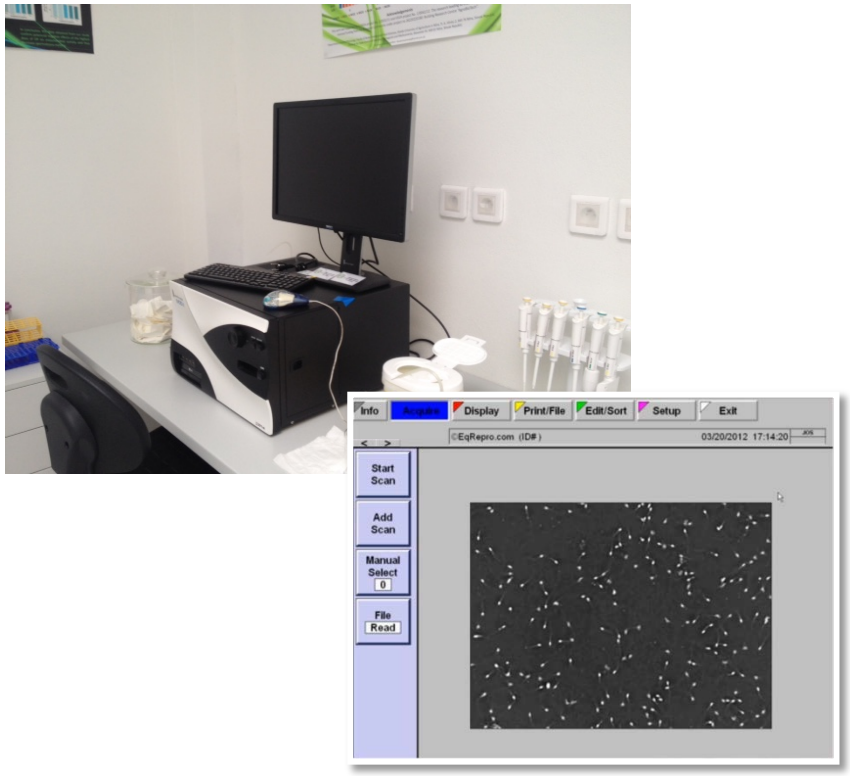
Sample Collection and Processing

- 10 semen samples from 8 breeding bulls
- Minimum progressive motility 70 % and sperm concentration 1×10^9 sperm/mL
- Two equal fractions diluted to $11x10^6$ sperm/mL
- Semen extender: Triladyl (egg yolk, TRIS, citric acid, sugar, buffers, glycerol and antibiotics) and diluted with distilled water
- Experimental group: the extender contained 1.5 mmol/L LYC
- Diluted semen samples → 0.25 mL French straws → 4 °C in 2 h → freezing at a pre-programmed rate in a digital freezing machine



Spermatozoa Motility Analysis

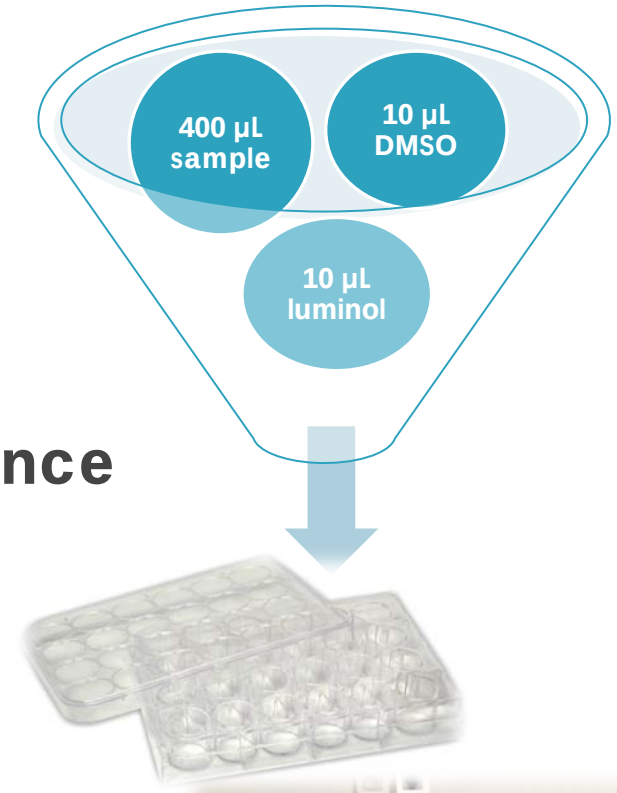
Computer assisted semen analysis (CASA) system



Experimental procedure:

- 10 µL sample: Makler counting chamber
- 10 microscopic fields subjected to each analysis in order to include at least 300 cells
- Spermatozoa motility: motion > 5µm/s (%)

Reactive Oxygen Species (ROS) Generation

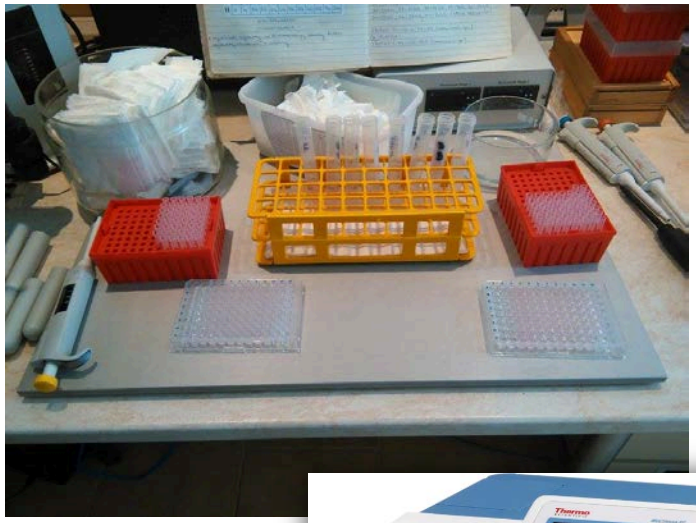


- Luminol-based chemiluminiscence
- 15 repeated cycles of 1 min

Negative controls = sperm-free cryomedium/PBS + luminol

Positive controls = sperm-free cryomedium/PBS + H_2O_2 + luminol

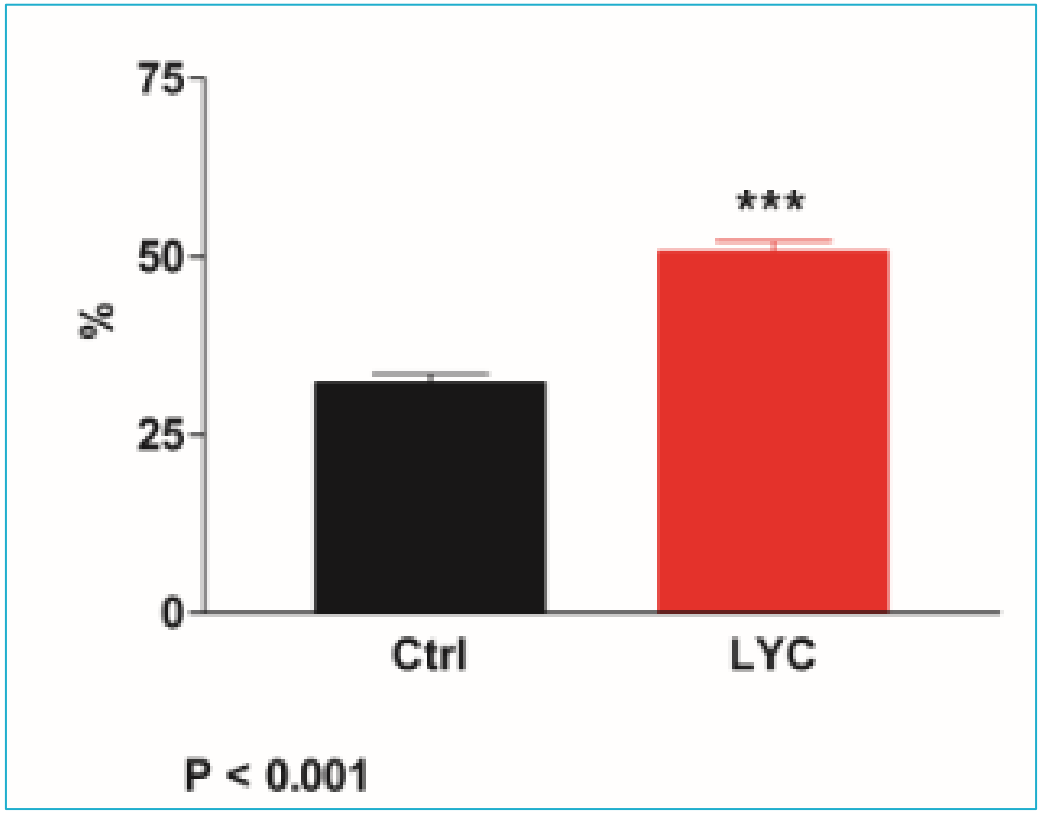
Lipid Peroxidation Assessment



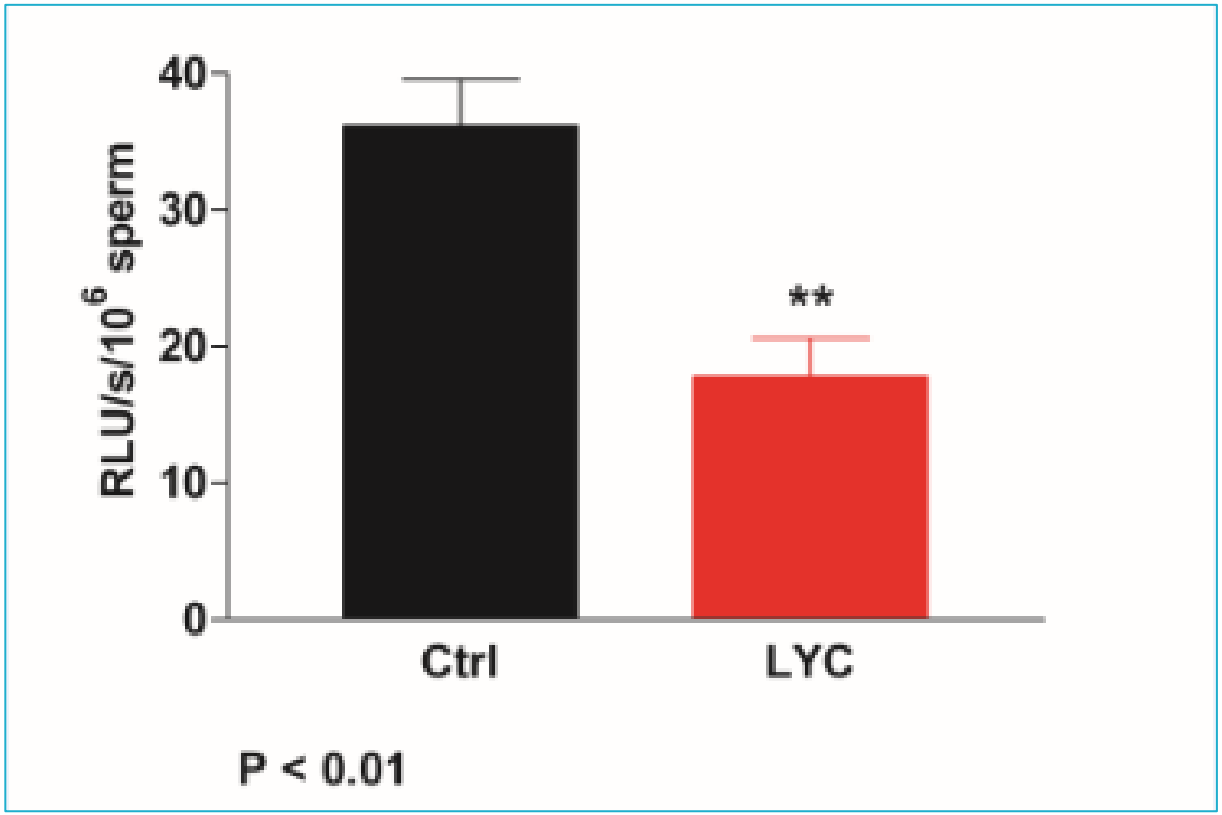
TBARS Method

- 100 µL sample + 4ml thiobarbituric acid (53%) dissolved in acetic acid (20%)
- Water bath: 1 hod. (100°C)
- Centrifugation: 8min/1800xg/4°C
- Plate reader: 530-540 nm

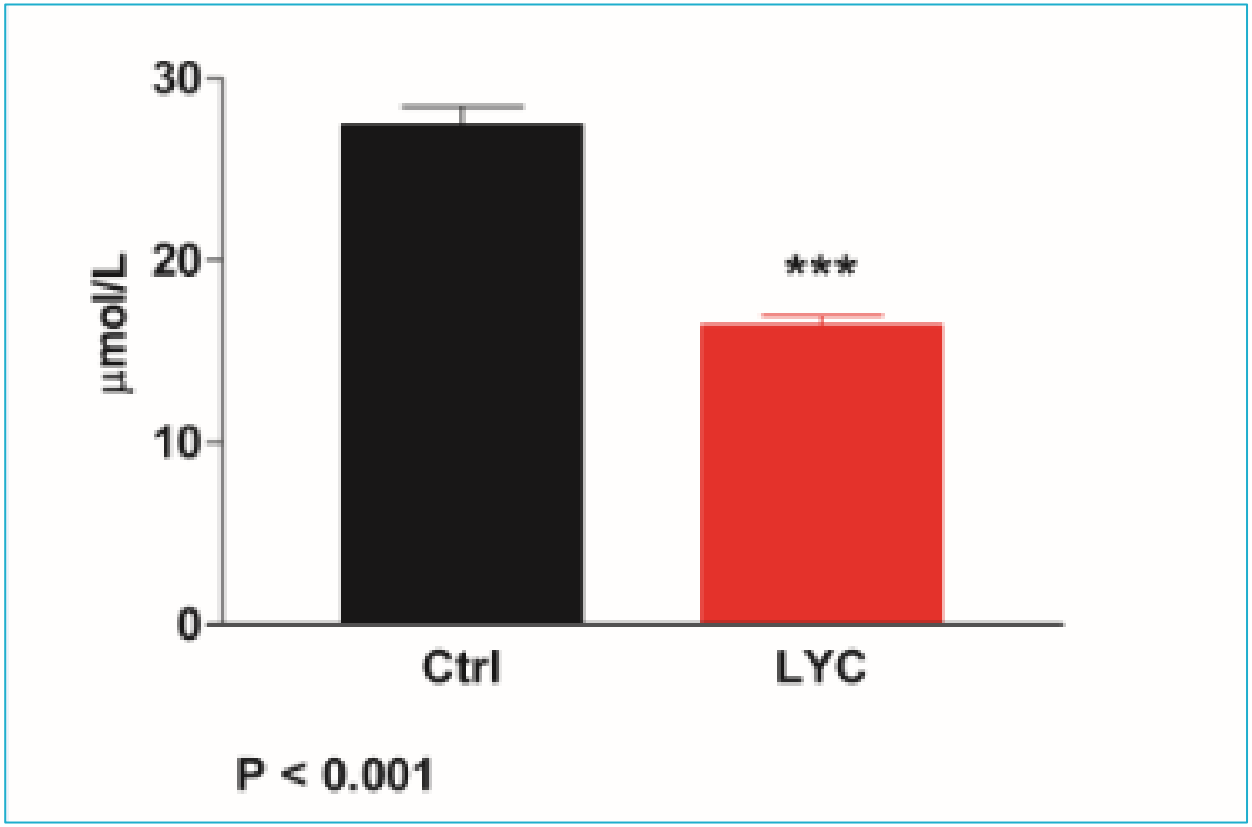
RESULTS: Spermatozoa Motility



RESULTS: ROS Production

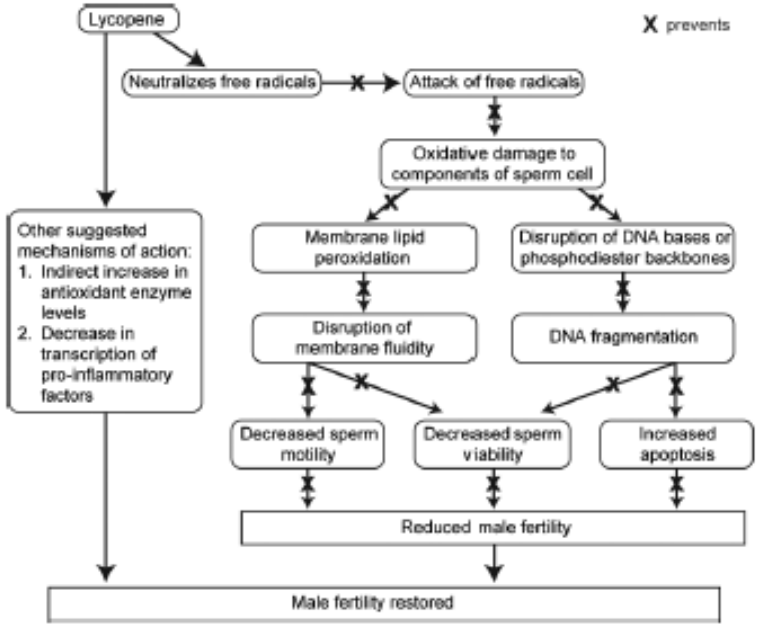


RESULTS: Lipid Peroxidation



LYCOPENE EXHIBITS PROTECTIVE EFFECTS ON CRYOPRESERVED SPERMATOZOA

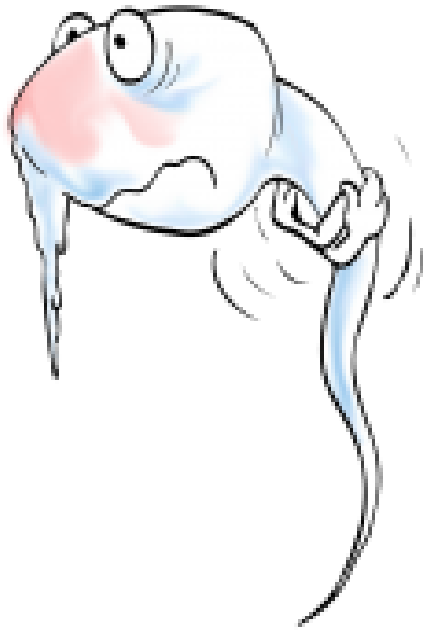
- Lower ROS generation
- Protection of membrane integrity
- Stimulation of spermatozoa motility



Potential practical application of the results

- Cryoconservation of mammalian, avian and reptilian spermatozoa
- Preservation of other somatic or reproductive cells and/or tissues
- Protection of genetic resources

THANK YOU FOR YOUR ATTENTION



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