PhD School in Veterinary Sciences - XXXII Cycle – Curriculum: Basic Sciences
Tutor: prof. Maria Laura Bacci Candidate: dott. Alberto Elmi

Responsable use of antibiotics in Swine Reproduction:
alternative methods to decrease bacterial contamination of A.I. doses

Phase I: Dose-effect studies of some essential oils on swine spermatozoa

INTRODUCTION

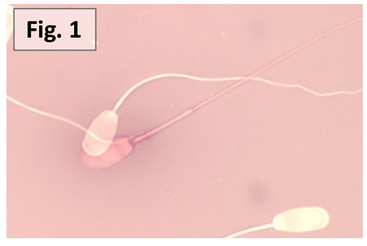
Essential oils (EOs) are products of the secondary metabolism of aromatic plants and are complex mixtures of several compounds. EOs show a wide variety of biological activities widely exploited in both Human and Veterinary Medicine. The pharmacologically active substances within EOs also show toxic effects when tested on different cell including human spermatozoa. Cytotoxicity studies on swine spermatozoa have two main outcomes: first, the identification of a cost-effective screening method for EOs evaluation in general, and then a direct application in reproductive medicine as alternative to the use of Antibiotics in artificial insemination (A.I.) doses.

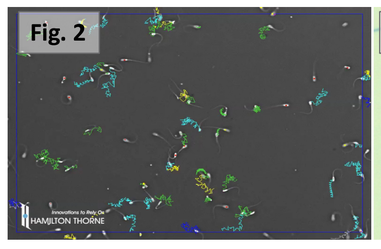
Thanks to the collaboration with dr. Maurizio Scozzoli (APA-CT, Forlì) we identified four Essential oils with possible antibacterial effects :

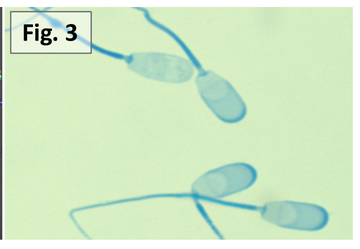
1) *Lavandula hybrida (La)*
2) *Rosmarinus officinalis (Ro)*
3) *Corydothimus capitatus (Cc)*
4) *Melaleuca alternifolia (Ma)*

MATERIALS & METHODS

1. The exact composition of each EO was tested by Gas Chromatography Flame Ionization Detector (GC-FID) (in collaboration with prof. Stefania Benvenuti, UNIMORE).
2. Each experimental sample was prepared by adding a fixed number of swine spermatozoa in a final volume of 5 ml of Swine Fertilization medium with 10 different dosages of EO (from 0.2 to 2mg/ml, at intervals of 0.2).
3. After 3 hours of incubation at 16°C (± 1°C), the samples were evaluated for the principal morpho-functional parameters: viability (Eosine-Nigrosine staining; **Fig.1**), objective motility (CASA; **Fig. 2**), acrosome status (Comassie blue staining; **Fig. 3**) and pH.
4. Moreover, for the highest and lowest dosages, the spermatic morphology was evaluated by Scanning Electron Microscopy (SEM) (in collaboration with prof. Annamaria Pisi, DipSA, UNIBO).







AIM

The aim of the phase I is to evaluate the dose-dependent effects of some essential oils on the main morpho-functional parameters of swine spermatozoa, in order to identify the highest dose, for each oil, that does not interfere with the quality of ejaculates. In phase II, the previously identified dosages will be tested for their antibacterial proprieties.

PRELIMINARY RESULTS

As of now 2 Eos, *Coridothymus capitatus* (Cc) and *Rosmarinus officinalis* (Ro), were tested on three ejaculates (n=3) collected from the same boar. The data regarding the acrosome status are currently under evaluation. The pH remained stable (6,8±0,12) throughout all of the experimental trials, thus it is not reported in this section.

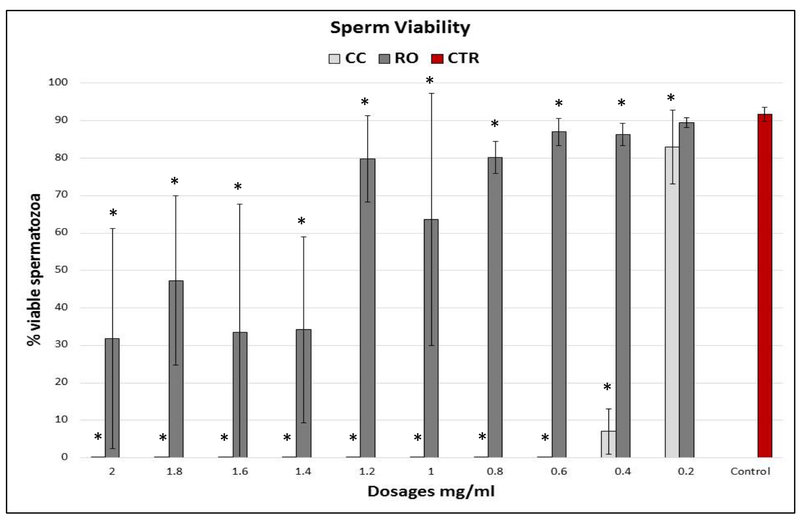


Table I : Sperm viability.
* = statistical difference ($p < 0.05$) between Control and treatment (T student test)

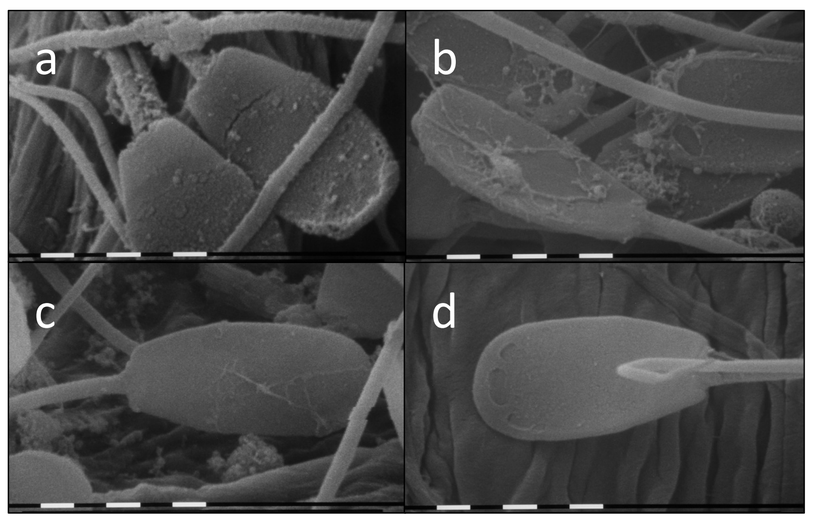


Figure 4: Spermatozoa Scanning Electron Microscopy (SEM, 10000x).
a) 2 mg/ml of Cc; b) 2 mg/ml of Ro; c) Capacitated spermatozoa;
d) Control

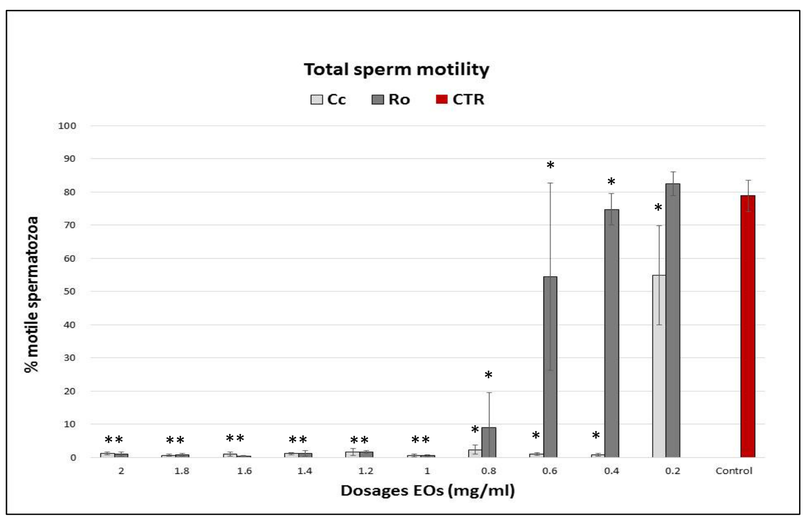


Table II: Total sperm motility ; CASA.
* = statistical difference ($p < 0.05$) between Control and treatment (T student test)

Papers:
- Romagnoli, Noemi; Lambertini, Carlotta; Ventrella, Domenico; Floriano, Dario; Elmi, Alberto; Barone, Francesca; Bacci Maria L., Ultrasound guided spinal catheter insertion in piglet: preliminary results, «VETERINARY ANAESTHESIA AND ANALGESIA», 2017, in press.
- Ventrella, Domenico; Dondi, Francesco; Barone, Francesca; Serafini, Federica; Elmi, Alberto; Giunti, Massimo; Romagnoli, Noemi; Forni, Monica; Bacci, Maria L., *The biomedical piglet: establishing reference intervals for haematology and clinical chemistry parameters of two age groups with and without iron supplementation*, «BMC VETERINARY RESEARCH», 2017, 13, pp. 1 – 8.

Oral comunication:
- Elmi, A.; Ventrella, D.; Barone, F.; Scozzoli, M.; Bacci, M.L., *Valutazione di effetti comparati di alcuni olii essenziali sulla funzionalità e integrità di spermatozoi suini.*, in: Natural 1, 2016, pp. 50 - 50 (atti di: IV Congresso Nazionale della SIROE, Roma - TOR Vergata, 25-26 Novembre 2016)

Poster:
- Ventrella, D.; Elmi, A.; Barone, F.; Scozzoli, M.; Bacci, M. L., *Prove preliminari per l'utilizzo di olii essenziali nel seme suino come alternativa agli antibiotici.*, in: Natural 1, 2016, pp. 43 - 43 (atti di: IV Congresso Nazionale della Società Italiana per la Ricerca sugli Oli Essenziali, Roma, 25-26 novembre 2016)
- Lambertini, C; Elmi, A.; Romagnoli, N.; Ventrella, D.; Barone, F.; Bacci, M. L., *Retrospective evaluation of the refinement of the spinal catheter placement techniques in piglets. Preliminary results*, in: ESLAV-ECLAM Annual Scientific Meeting on animal welfare. Abstract book, 2016(atti di: ESLAV-ECLAM Annual Scientific Meeting on animal welfare, LIONE, FRANCIA, 15-18 NOVEMBRE 2016)
- Francesca, Barone; Domenico, Ventrella; Alberto, Elmi; Maria Laura, Bacci, *The challenges in establishing HEP in laboratory swine*, in: ESLAV-ECLAM 2016 Annual Scientific Meeting on Animal Welfare, 2016, pp. 83 - 85 (atti di: ESLAV-ECLAM 2016 Annual Scientific Meeting on Animal Welfare, Lione, Francia, 15-18.11.2016)