

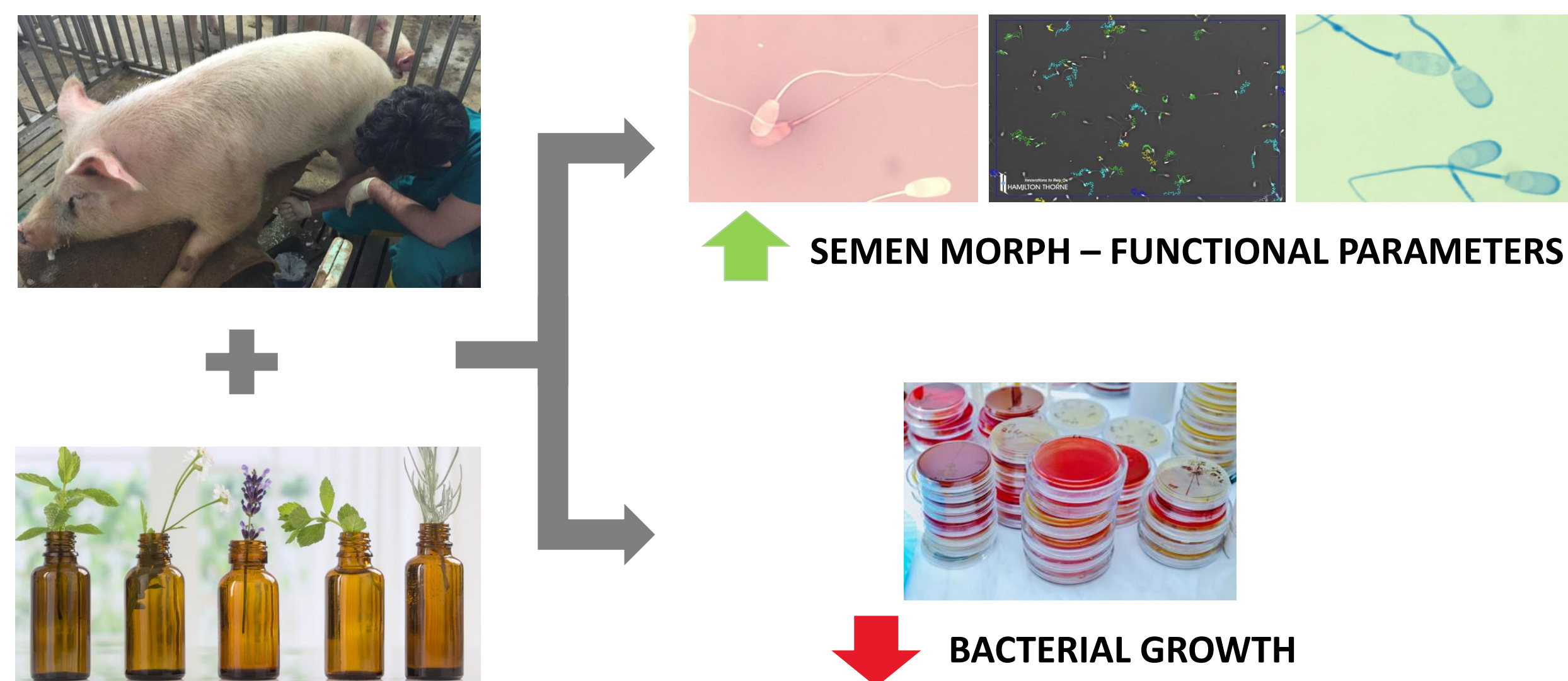


Responsible use of antibiotics in Swine Reproduction: alternative methods to decrease bacterial contamination of A.I. doses

INTRODUCTION:

Traditional medicine, since the dawn of history, has exploited the knowledge about plants and natural compounds for medical treatments, laying the foundations of modern medicine and pharmacology. The growing interest towards the application of natural compounds, and in particular essential oils, stems from their potential multi-purpose functional use as antibacterial, antiviral, antifungal, and antioxidant agents. During the liquid phase storage of the seminal material, refrigerated at 15-17°C, the bacterial growing can determine alteration of the spermatozoa quality, reducing the viability and motility, in addition to the risk of female's reproductive disorders and embryonic/fetal death. Furthermore, the presence of pathogens cannot be excluded. For all of these reasons, the antimicrobials are added to the semen extenders and their presence is legislated by the European Directive 90/429. Nonetheless, antimicrobial resistance is increasing in the porcine productions with high risks also for human, driving the need to alternative strategies for bacterial growth control.

GRAFICAL AIMS:



CONCENTRATION – EFFECT STUDIES OF SOME ESSENTIAL OILS



Article

Thymbra capitata (L.) Cav. and *Rosmarinus officinalis* (L.) Essential Oils: In Vitro Effects and Toxicity on Swine Spermatozoa

Alberto Elmi ¹, Domenico Ventrella ^{1,*}, Francesca Barone ¹, Gianfranco Filippini ², Stefania Benvenuti ³, Annamaria Pisi ², Maurizio Scozzoli ⁴ and Maria L. Bacci ¹



Article

In Vitro Effects of Tea Tree Oil (*Melaleuca Alternifolia* Essential Oil) and its Principal Component Terpinen-4-ol on Swine Spermatozoa

Alberto Elmi ¹, Domenico Ventrella ^{1,*}, Francesca Barone ^{1,2}, Giacomo Carnevali ¹, Gianfranco Filippini ³, Annamaria Pisi ³, Stefania Benvenuti ⁴, Maurizio Scozzoli ⁵ and Maria Laura Bacci ¹



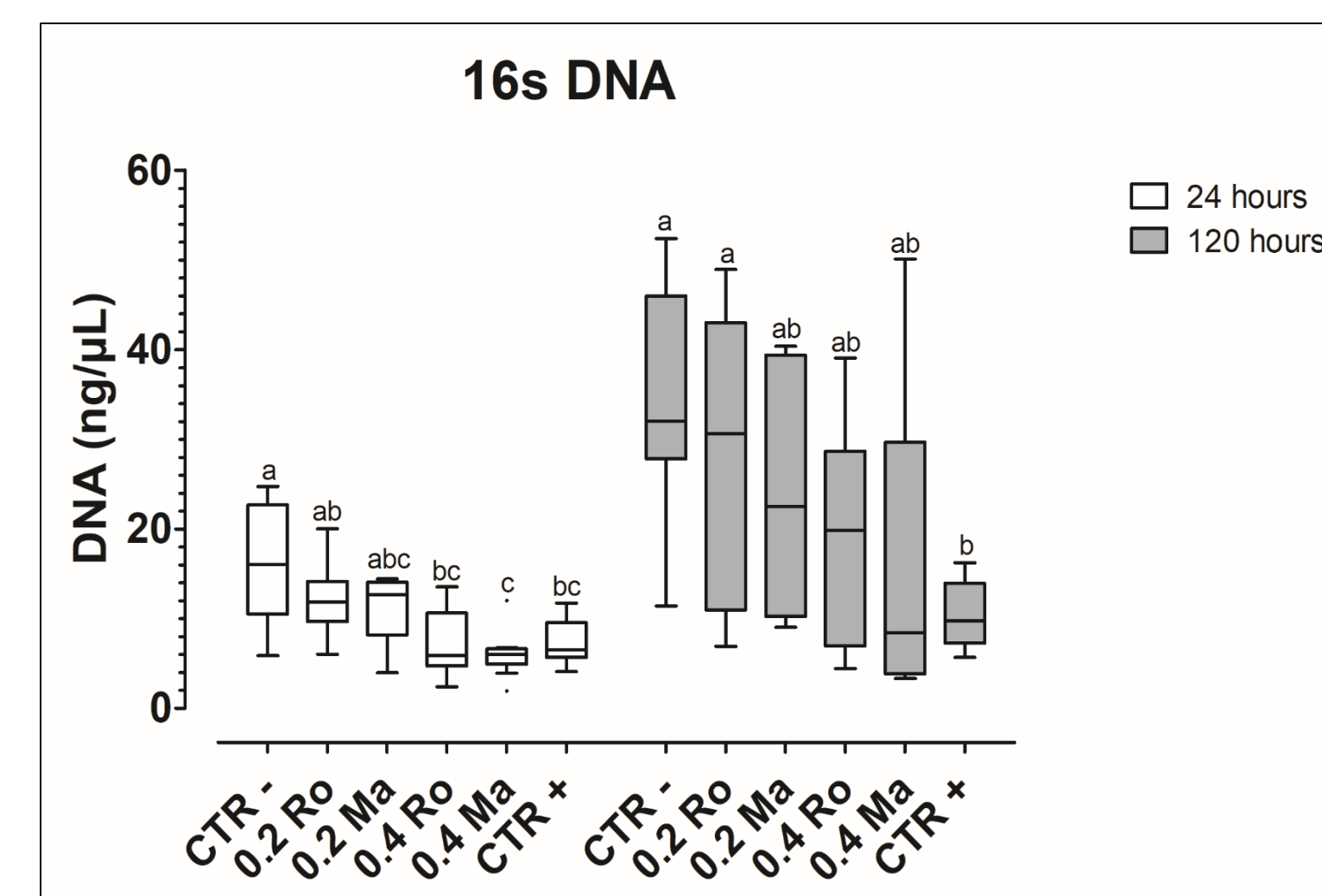
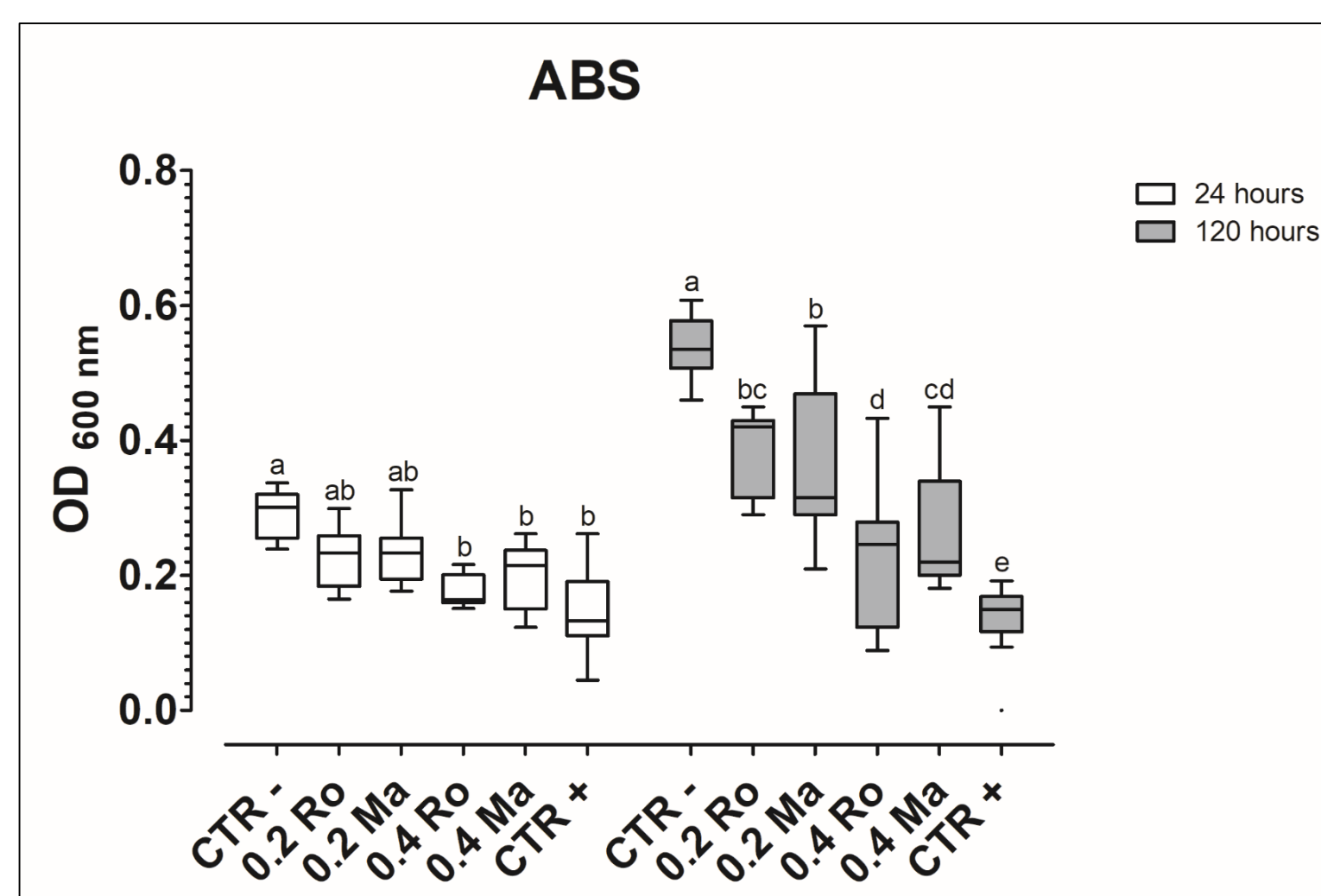
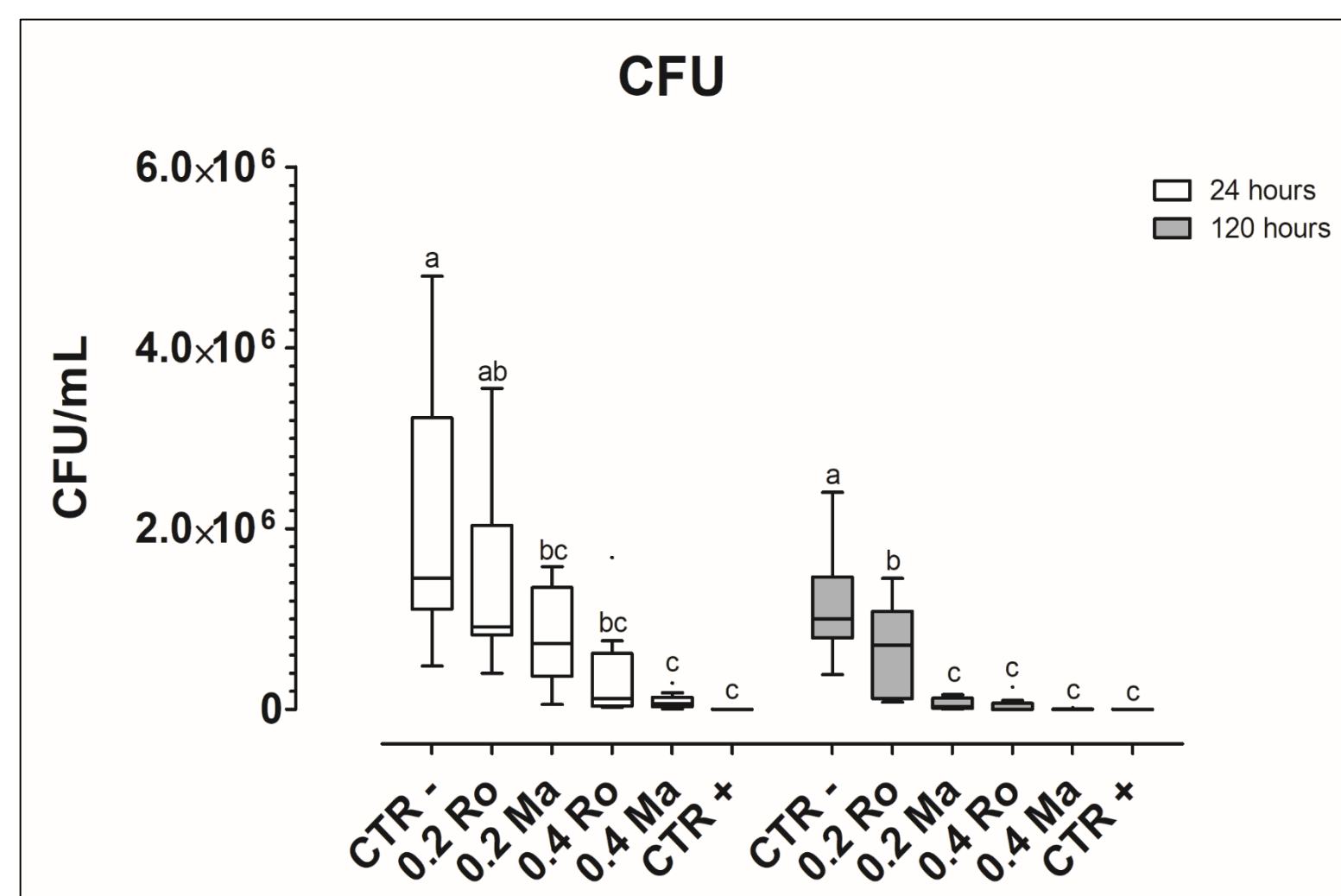
M. alternifolia AND *R. officinalis* ESSENTIAL OILS DECREASE BACTERIAL GROWTH IN SEMINAL PLASMA REFRIGERATED AT 16°C

In the present study, sperm poor fractions from 9 ejaculates (3 from each boar) were used. After collection, they were immediately centrifuged (3000 g x 20 minutes) to separate spermatozoa pellet from the supernatant to obtain the seminal plasma (SP). The experimental samples were prepared with 50% of SP and 50% of Swine Fertilization Medium (SFM), and added with 3x10⁷ CFU/mL *Escherichia coli* DH5a strain (cod. C2007-1, Clontech Lab. Inc, CA, USA). Two different essential oils (EOs) were tested: *Melaleuca alternifolia* and *Rosmarinus officinalis*, both at two different concentrations (0.2 mg/mL and 0.4 mg/mL), according to the spermatozoa toxicity studies previously carried out by the authors. Two different control samples were prepared: added with Ampicillin (500 mg/L) (CTR +) or without any antibiotic (CTR -).

The experimental samples were incubated for 5 days in a refrigerated bath a 16°C (±1 °C) and samples were harvested at 24 hours and 5 days (120 hours).

Bacterial levels were tested in every sample by means of cultural assays (IZSLER, section PARMA), optical density at 600 nm (OD600) using spectrophotometer, and 16s DNA quantification by Real Time PCR (BSBT-BCM). To evaluate differences between the treatment groups at the two time points, repeated measures ANOVA was performed with the significance level set at 0.05. Post hoc analyses were performed for each time point (24h and 120h) by means of Tukey's test (p < 0.05)

PRELIMINARY RESULTS:



CTR - = control without antibiotics; 0.2 Ro = 0,2 mg/mL *R. officinalis*; 0.4 Ro = 0,4 mg/mL *R. officinalis*; 0.2 Ma = 0,2 mg/mL *M. alternifolia*; 0.4 Ma = 0,4 mg/mL *M. alternifolia*; CTR + = control with antibiotics. Different letters indicate statistical differences between groups at each time point (p < 0,05).

CONCLUSION:

Overall, the result of the different phases of this project seem to suggest the feasibility of Essential Oils as substituted for conventional antibiotics. Indeed, for the tested oils, the non-toxic concentrations still show efficacy in reducing replication of bacteria. Nonetheless, the current laws regarding the commercialization of swine AI doses still demand the use of conventional antibiotics. Further studies will potentially strengthen such hypothesis and maybe drive a change in legislation.

Papers:

- Elmi A, Ventrella D, Luca Laghi, Carnevali G, Zhu C, Pertile G, Barone F, Benfenati F, Bacci ML. «1H NMR Spectroscopy Characterization of Porcine Vitreous Humor in Physiological and Photoreceptor Degeneration Conditions» *INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE*, 2019, 60, pp. 741 – 747
- Elmi A, Ventrella D, Barone F, Carnevali G, Filippini G, Benvenuti S, Pisi A, Scozzoli M, Bacci ML. «In Vitro Effects of Tea Tree Oil (*Melaleuca Alternifolia* Essential Oil) and its Principal Component Terpinen-4-ol on Swine Spermatozoa» *MOLECULES*, 2019, 24, pp. 1 – 15
- Ventrella D, Elmi A, Barone F, Carnevali G, Govoni N, Bacci ML. «Hair Testosterone and Cortisol Concentrations in Pre- and Post-Rut Roe Deer Bucks: Correlations with Blood Levels and Testicular Morphometric Parameters» *ANIMALS*, 2018, 8, pp. 1 – 9
- Barone F, Nannoni E, Elmi A, Lambertini C, Gerardi Scorpio D, Ventrella D, Vitali M, Maya-Vetencourt JF, Martelli G, Benfenati F, Bacci ML. «Behavioral Vision Assessment in the Biomedical Pig» *JOURNAL OF AMERICAN ASSOCIATION OF LABORATORY ANIMALS*, 2018, 57, pp. 350 – 356
- Elmi A, Banchelli F, Barone F, Fantinati P, Ventrella D, Forni M, Bacci ML. «Semen evaluation and in vivo fertility in a Northern Italian pig farm: Can advanced statistical approaches compensate for low sample size? An observational study» *ANIMAL REPRODUCTION SCIENCE*, 2018, 192, pp. 61 – 68.
- Elmi A, Ventrella D, Barone F, Filippini G, Benvenuti S, Pisi A, Scozzoli M, Bacci ML. «*Thymbra capitata* (L.) Cav. and *Rosmarinus officinalis* (L.) Essential Oils: In Vitro Effects and Toxicity on Swine Spermatozoa» *MOLECULES*, 2017, 22, pp. 2162 – 2173.
- Romagnoli N, Lambertini C, Ventrella D, Floriano D, Elmi A, Barone F, Bacci ML. «Ultrasound guided spinal catheter insertion in piglet: preliminary results» *VETERINARY ANAESTHESIA AND ANALGESIA*, 2017, Volume 44, Issue 6, November 2017, pp. 1391-1396.
- Ventrella D, Dondi F, Barone F, Serafini F, Elmi A, Giunti M, Romagnoli N, Forni M, Bacci ML. «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.
- Ventrella D, Laghi L, Barone F, Elmi A, Romagnoli N, Bacci ML. «Age-Related 1H NMR Characterization of Cerebrospinal Fluid in Newborn and Young Healthy Piglets» *PLOS ONE*, 2016, 11, pp. 1 - 13