

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

Dose-effect studies of some essential oils on swine spermatozoa



Article

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

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Essential oils possess a variety of biological properties (i.e., antioxidant, antibacterial, and cytotoxic) that could possibly be applied in reproductive medicine, but their effects on spermatozoa are still partially unknown. The aim of the study was to describe the effects of *Thymbra capitata* (L.) Cav. and *Rosmarinus officinalis* (L.) essential oils on the main morpho-functional parameters of swine spermatozoa. Essential oils were preliminary characterized by gas chromatography and added with emulsifiers to facilitate diffusion. Experimental samples were prepared by suspending a fixed number of spermatozoa in 5 mL of medium with 10 different concentrations of essential oil (0.2–2 mg/mL, at intervals of 0.2). After 3 h of incubation, samples were analyzed for pH, viability, objective motility, and acrosome status.

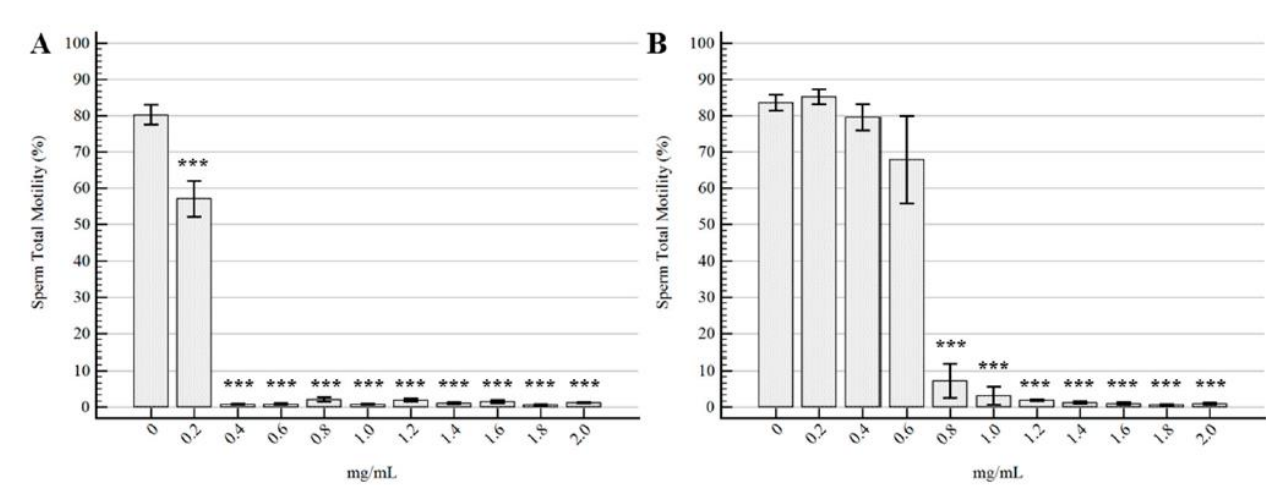


Figure 2. Effects of the EOs on total motility. (A) *Thymbra capitata*. (B) *Rosmarinus officinalis*. Data are expressed as the mean \pm standard error of the mean ($n = 6$), and 0 mg/mL represents the control sample (only emulsifiers). *** = $p < 0.001$.

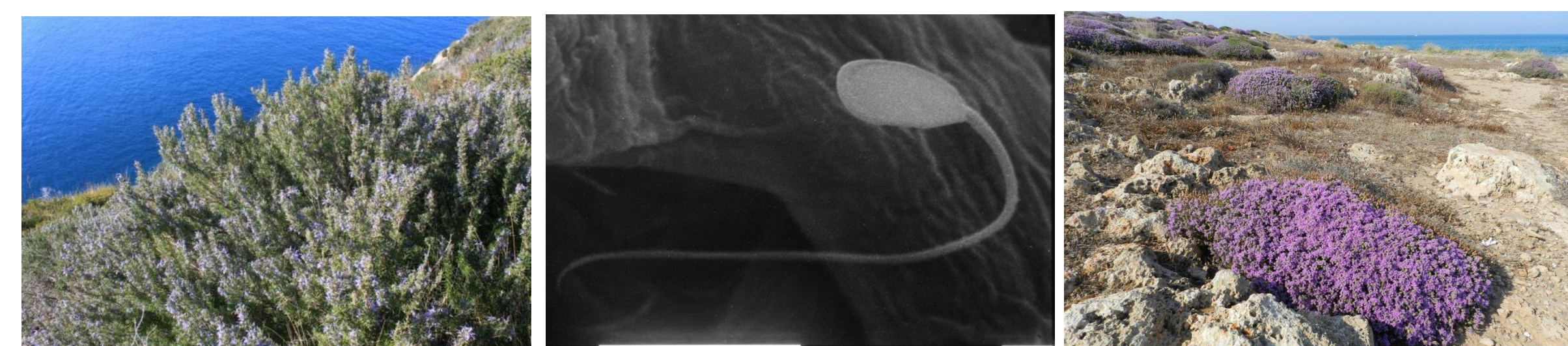


Figure 4. Scanning electron micrographs on the effect of EOs on sperm morphology. Semen samples (a) treated with 0.2 mg/mL of *Thymbra capitata*; (b) with 0.2 mg/mL of *Rosmarinus officinalis*; (c) with 2 mg/mL of *Tc*; (d) with 2 mg/mL of *Ro*; (e) capacitated spermatozoa; and (f) control sample (bars = 1 μ m).

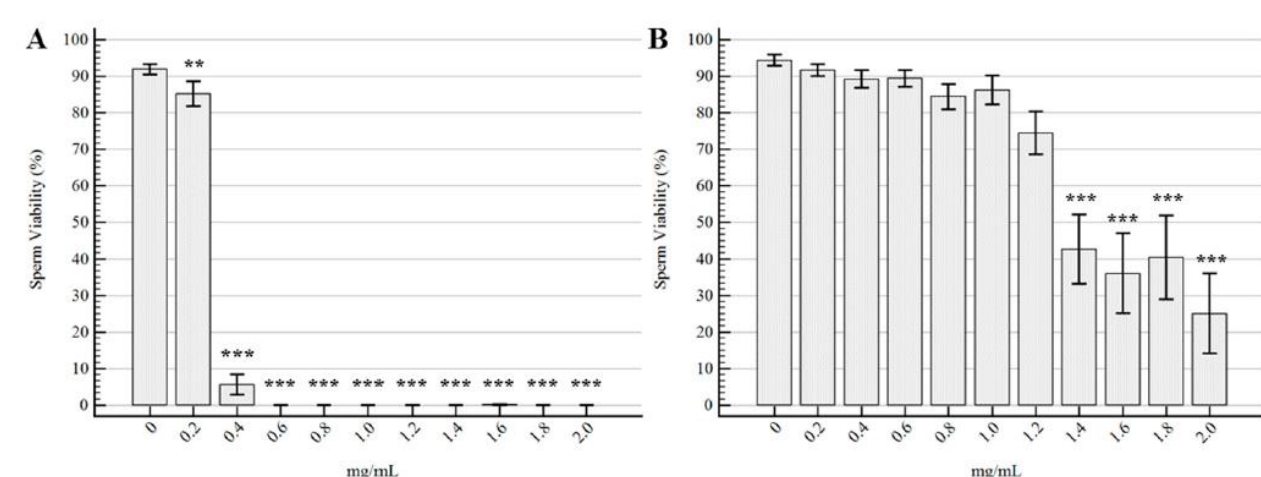


Figure 1. Effects of the EOs on sperm viability. (A) *Thymbra capitata*; (B) *Rosmarinus officinalis*. Data are expressed as mean \pm standard error of the mean ($n = 6$). 0 mg/mL represents the control sample (only emulsifiers). ** = $p < 0.01$; *** = $p < 0.001$.

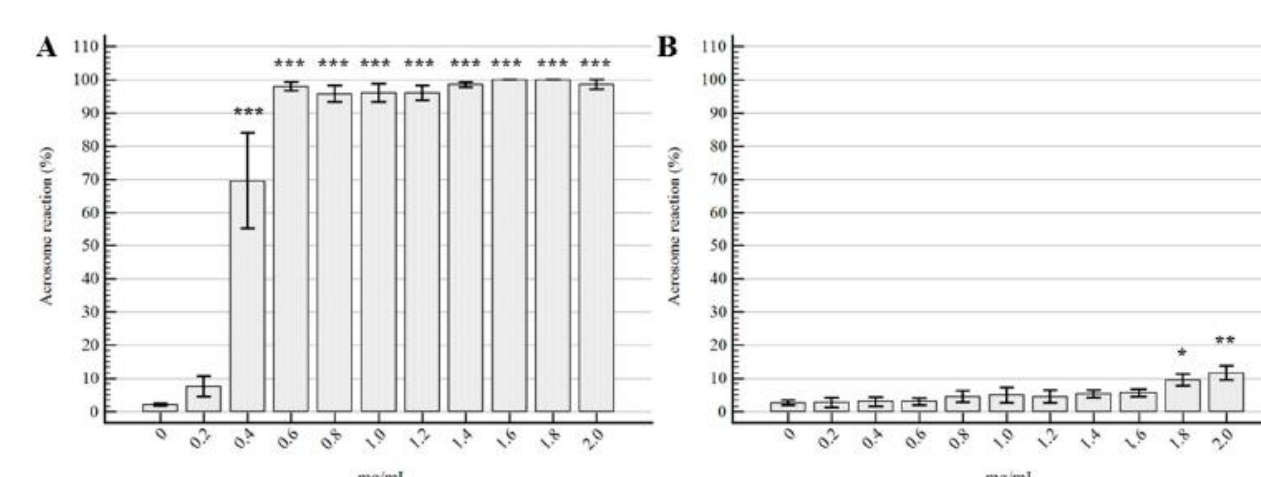
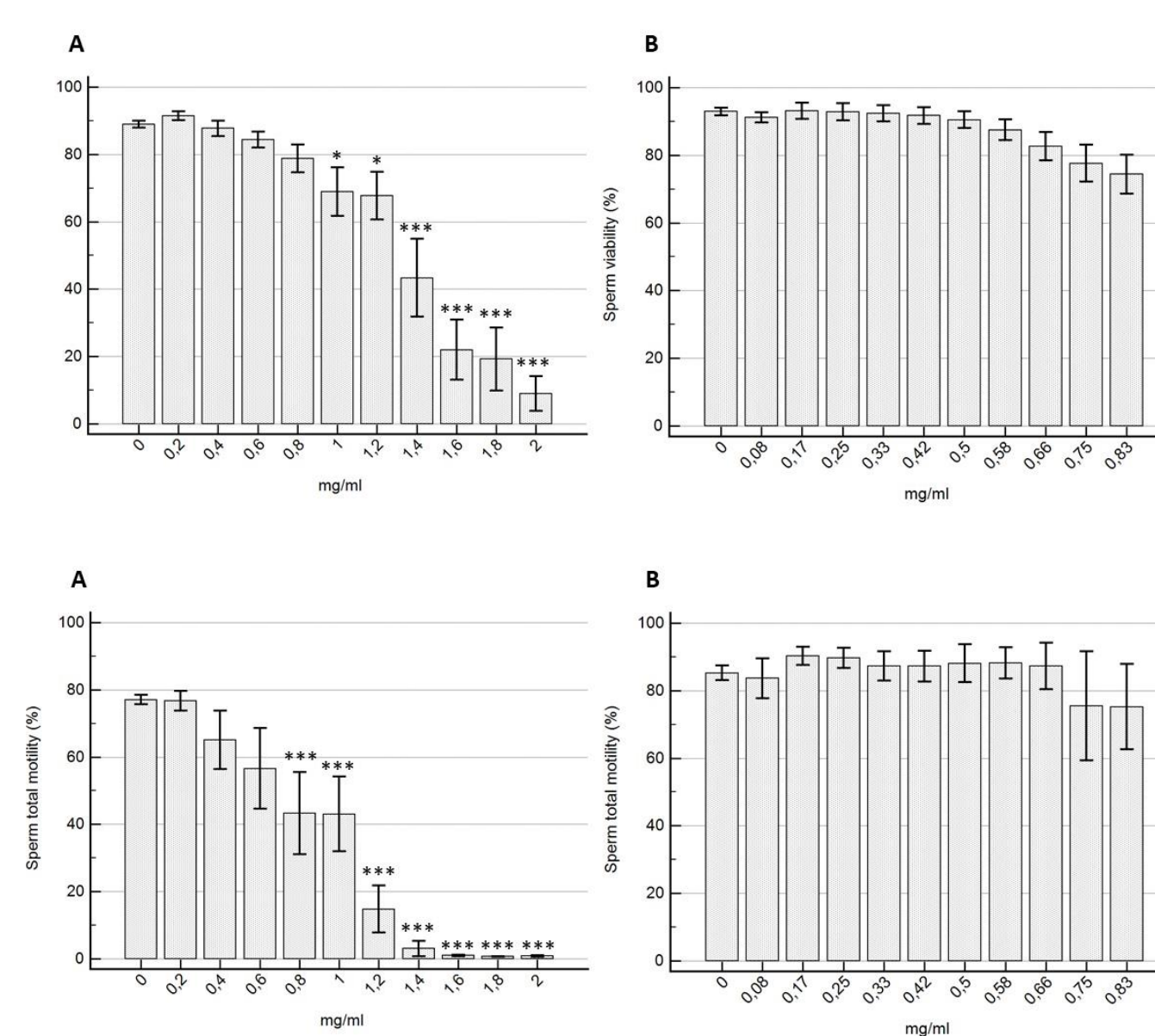


Figure 3. Effects of the EOs on acrosome status. (A) *Thymbra capitata*; (B) *Rosmarinus officinalis*. Data are expressed as mean \pm standard error of the mean ($n = 6$). 0 mg/mL represents the control sample (only emulsifiers). * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

PRELIMINARY RESULTS: *Melaleuca alternifolia* and Terpinen-4-ol

The aim of the study was to evaluate the effects of *Melaleuca alternifolia* essential oil (EO) and its principal compound, the Terpinen-4-ol, on the principal morpho-functional parameters of swine spermatozoa. Terpinen-4-ol seems to play a key role in mediating antimicrobial activity of the *M. alternifolia* essential oil. The experimental protocol was previously validated and described by the authors: briefly, after Gas Chromatography characterization of the EO, experimental samples were prepared by suspending a fixed number of spermatozoa in 5 mL of swine fertilization medium (SFM) with 10 different concentrations of EO. After 3 hours of incubation at 16°C ($\pm 1^\circ\text{C}$), the samples were evaluated for Viability (Eosine-Nigrosine staining), Objective Motility (CASA), Acrosome Status (Comassie blue staining) and pH. The same experimental protocol was applied for terpinen-4-ol, adjusting the tested concentrations to its presence in the used EO (41,5 %).



A = *Melaleuca alternifolia* ($n=8$); B = Terpinen-4-ol ($n=3$); * = $p < 0,05$; *** = $p < 0,001$;



M. Alternifolia EO

All parameters, with the exception of pH, resulted statically significant to the variance analysis. Acrosome status was significantly altered starting from 1,4 mg/ml ($p < 0,0001$), MMP from 1,2 mg/mL ($p < 0,0001$) and Viability from 1 mg/ml ($p = 0,0370$). Total motility, the most sensitive parameter as already reported by literature, was altered starting from 0,8 mg/mL ($p = 0,0003$).

Terpinen-4-ol

The preliminary results, deriving from a small amount of experiments ($n=3$), did not highlight any statistical difference between the treated samples and the control ones. Nonetheless, a decreasing trend in the quality of spermatozoa can be noticed for the higher concentrations.

ACHIEVEMENTS

The results of the toxicity studies allowed for the identification of EO concentrations well tolerated by swine spermatozoa: up to 0,6 mg/ml for *Rosmarinus officinalis* and up to 0,8 mg/ml for *Melaleuca alternifolia*. Such concentrations will be used for further studies. Terpinen-4-ol does not seem to have effects on the spermatozoa, despite being the most active compound of *M. alternifolia* according to literature. On the other hand, *Thymbra capitata* resulted to be toxic for spermatozoa, thus will be excluded from the future studies. Nonetheless, the latter may be proposed as a spermicidal agent.

Papers:

- 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, accepted.
- 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

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