

# REGENERATIVE AND ANTIMICROBIAL BENEFITS OF INTRAUTERINE OZONE THERAPY DURING THE PUERPERIUM IN COWS: IN VITRO AND IN VIVO FINDINGS.

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## RESUMO

**Introduction:** Puerperal uterine disorders are a main cause of impaired fertility in cattle. Approximately 90% of the cows are affected by infectious endometritis during the first weeks after calving. Ozonized vegetable oils have been used as therapeutic stressors and powerful oxidizers. Therefore, the goals of the present study were: a) To describe the endometrial microscopic changes induced by the uterine infusion of ozonized sunflower oil in post-partum primiparous cows, and b) to determine the *in vitro* antimicrobial potential of ozonized sunflower oil against bacteria isolated from the uterus of post-partum cows. **Material and Methods:** Fourteen primiparous cows received a single infusion of ozonized or non-ozonized sunflower oil (O<sub>3</sub>-oil and control groups, respectively; n=7 cows/group) between days 11 and 13 after calving. Light and Scanning electron microscopy analyses were performed immediately before and 15 days after treatment (D0 and D15, respectively). In addition to the histomorphology, four histomorphometry parameters were measured: Endometrial epithelium height, glandular epithelium height, glandular diameter and glandular luminal area. Antibiotic sensitivity testing (AST), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ozonized and non-ozonized oils against bacteria (*Streptococcus* spp., *Staphylococcus* spp., *Escherichia coli* and *Arcanobacterium pyogenes*) isolated from the uterus of cows were determined. **Results:** Independently of the group ( $P>0.1$ ), the glandular epithelium height and the glandular diameter did not change ( $P>0.1$ ). the control group showed an 8-fold increase ( $P<0.01$ ) for glandular luminal area associated to diffuse endometrial hyperemia, hemorrhagic spots and dilated endometrial vessels. In opposite, endometrial glandular dilatations and morphological findings associated to degeneration were not found in cows treated with ozonized oil (O<sub>3</sub>-oil). Moreover, scanning electron microscopy indicated the absence of morphological abnormalities induced by the uterine infusion of ozonized sunflower oil. Endometrial samples from O<sub>3</sub>-oil and control group had specific microvilli-covered cells in D0 and D15. The cells were predominantly covered by microvilli. Around 30% of the observed fields presented a surface with less than 50% microvilli coverage. The AST indicated the sensitivity of all bacteria to the ozonized sunflower oil. Independently of the pathogen, the zone inhibitions were greater ( $P<0.05$ ) for ozonized oil than to Tetracycline. MIC and MBC of the ozonized sunflower oil were  $\leq 1.50\mu\text{g mL}^{-1}$ . Non-ozonized sunflower oil (control group) did not show antimicrobial properties (absence of zone inhibition in AST; MIC and MBC  $> 100\%$ ). **Conclusion:** The infusion of ozonized sunflower oil did not induce deleterious changes on the luminal surface of the uterus and endometrial gland morphology in post-partum cows. Local O<sub>3</sub> therapy also prevented the occurrence of glandular dilatation and chronic inflammation. Finally, ozonized sunflower oil had germicidal action against

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uterine bacteria.

**PALAVRAS-CHAVE:** bacteria, cattle, inflammation, integrative medicine, sunflower oil

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