TITLE: USE OF *MESO*-TETRAMETHYLPYRIDYL IN THE ANTIMICROBIAL PHOTODYNAMIC THERAPY AGAINST *Leptospira interrogans* Serovar Hardjo AND ITS ACTION ON BOVINE OOCYTES

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ABSTRACT:
Due to the growth of reproductive biotechnology, the concern with the sanity of herds in the face of diseases transmitted by semen or embryos has increased. *Leptospira interrogans* is a Gram-negative spirochete that leads to leptospirosis, disease that causes infertility in affected herds. Among several transmission routes, there is the risk by manipulation of embryos in vitro. Thus, as an alternative for the inactivation of infectious agents, the Antimicrobial Photodynamic Therapy (aPDT) has been gaining prominence as it does not cause microbial resistance. Combinating photosensitizer (PS), light and molecular oxygen the reactive oxygen species (ROS) are formed in high levels inducing cell death. The *meso*-tetramethylpyridyl with zinc (ZnTMPyP) is a cationic PS that has been extensively tested in aPDT. In this study, we examined the antimicrobial effect of ZnTMPyP against *Leptospira interrogans* Serovar Hardjo and its action on bovine oocytes. The culture was incubated with PS (10µM) without light; diluted in EMJH medium (1:4) and quantified in a spectrophotometer (OD 600nm). The samples were irradiated for 30min (intensity of 180mW/cm^2, halogen lamp 470 to 750nm), an aliquot of each sample was diluted in EMJH medium, incubated (29°C) for seven days and evaluated by dark field microscopy and spectrophotometer reading. The controls were: culture without PS/irradiation; culture without PS with irradiation; culture with PS without irradiation. Oocytes (CCO's) were aspirated from ovaries of slaughterhouse, classified and incubated in controlled incubator. After 22 hours, CCO's were irradiated for 30 min under equal conditions to leptospires, washed in ten drops of bench LAV medium and fertilized (FIV). The controls were: CCO's without PS/irradiation and CCO's without PS with irradiation. The structures were partially stripped, washed, transferred to culture medium and incubated for seven days. In D3, the structures were evaluated for cleavage and, in D7, as for the embryo rate. No significant difference was observed among the controls samples in both steps. Great cell destruction was noted in the culture samples irradiated with ZnTMPyP possibly due to excellent electrostatic interaction with phospholipids on the bacterial membrane surface. PS was not detrimental to the CCO's, evidenced by production of embryos. The ZnTMPyP can be used for the control of *Leptospira interrogans* and aPDT could prove to be a potential method for control of microorganisms in oocytes.

Keywords: aPDT, bovine reproduction, leptospirosis, photoinactivation, photosensitizer.

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