

Gene expression of the antioxidant enzyme Peroxiredoxin 1 (PRDX-1) from *in vitro* produced bovine embryos in culture media with essential oil of *Lippia origanoides*

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INTRODUCTION

The *in vitro* production (IVP) of bovine embryos is one of the most widespread reproductive biotechniques in Brazil in the last decade. Nevertheless, during IVP, the embryo is exposed to several external agents that generate excessive production of reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), which can modify biological molecules (lipids, proteins, and nucleic acids), inducing abnormal development and embryonic death. To circumvent this damage, the addition of antioxidants to the embryonic culture medium has become routine. In addition, antioxidants have the effect of directly influencing gene expression through genetic reprogramming, preventing DNA and histone methylation, and modulating the expression of antioxidant enzymes such as peroxiredoxins (PRDXs).

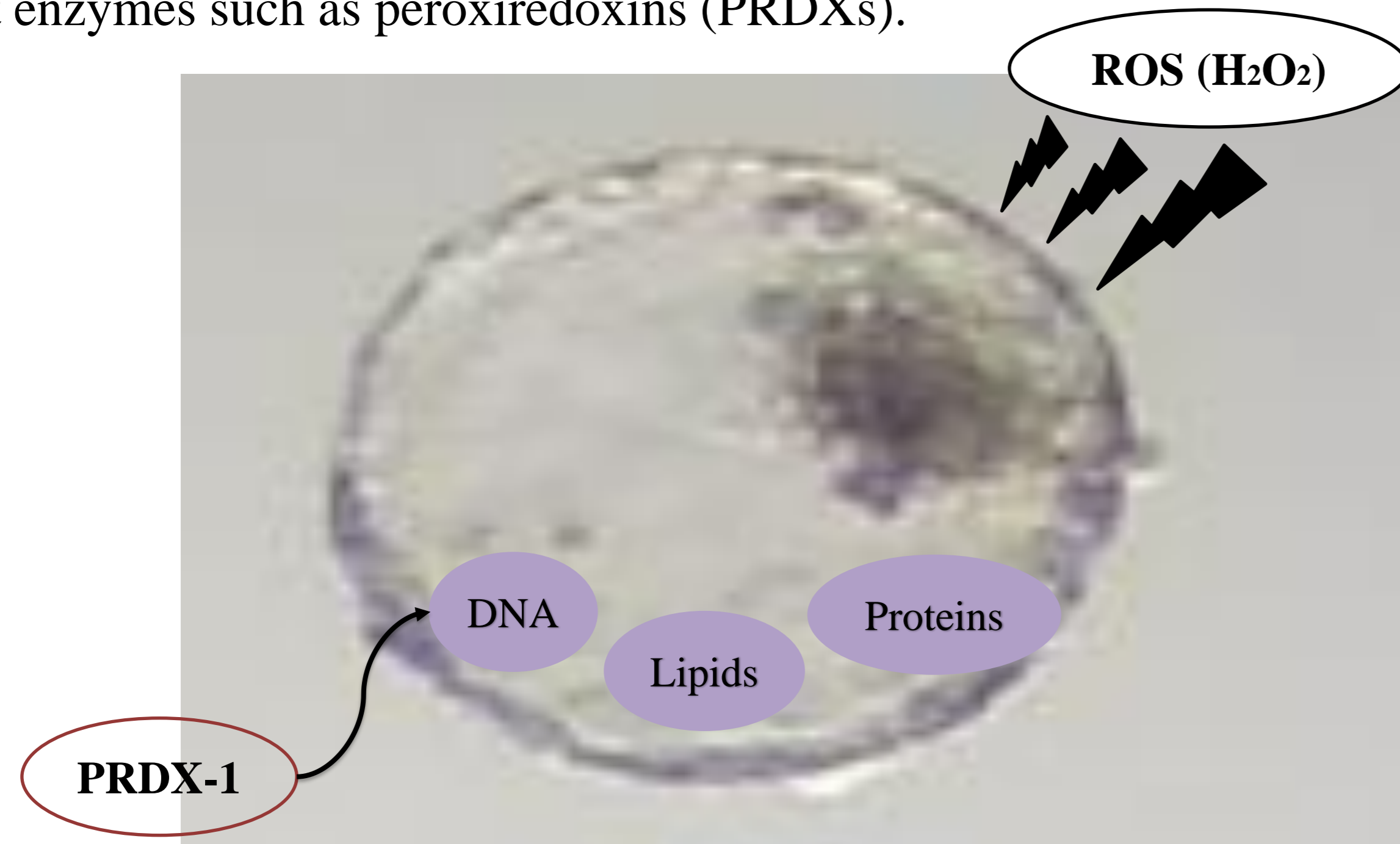


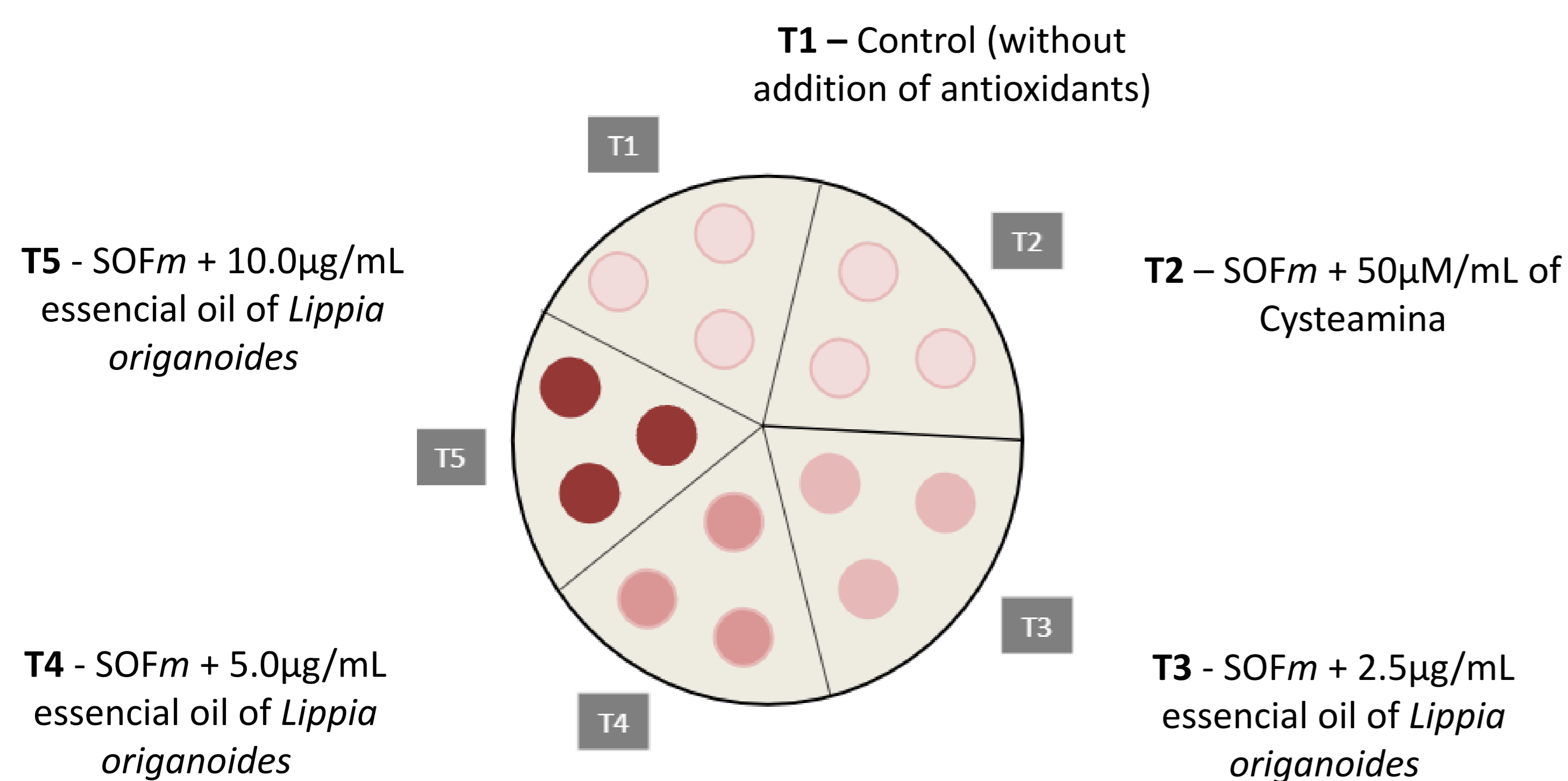
Figure 1. Bovine blastocysts cultured *in vitro* with *Lippia origanoides* (Personal archive)

OBJECTIVES

This study evaluated the influence of the addition of essential oil obtained from the plant *Lippia origanoides* to the *in vitro* bovine embryo culture medium on the gene expression of the antioxidant enzyme Peroxiredoxin 1 (PRDX-1).

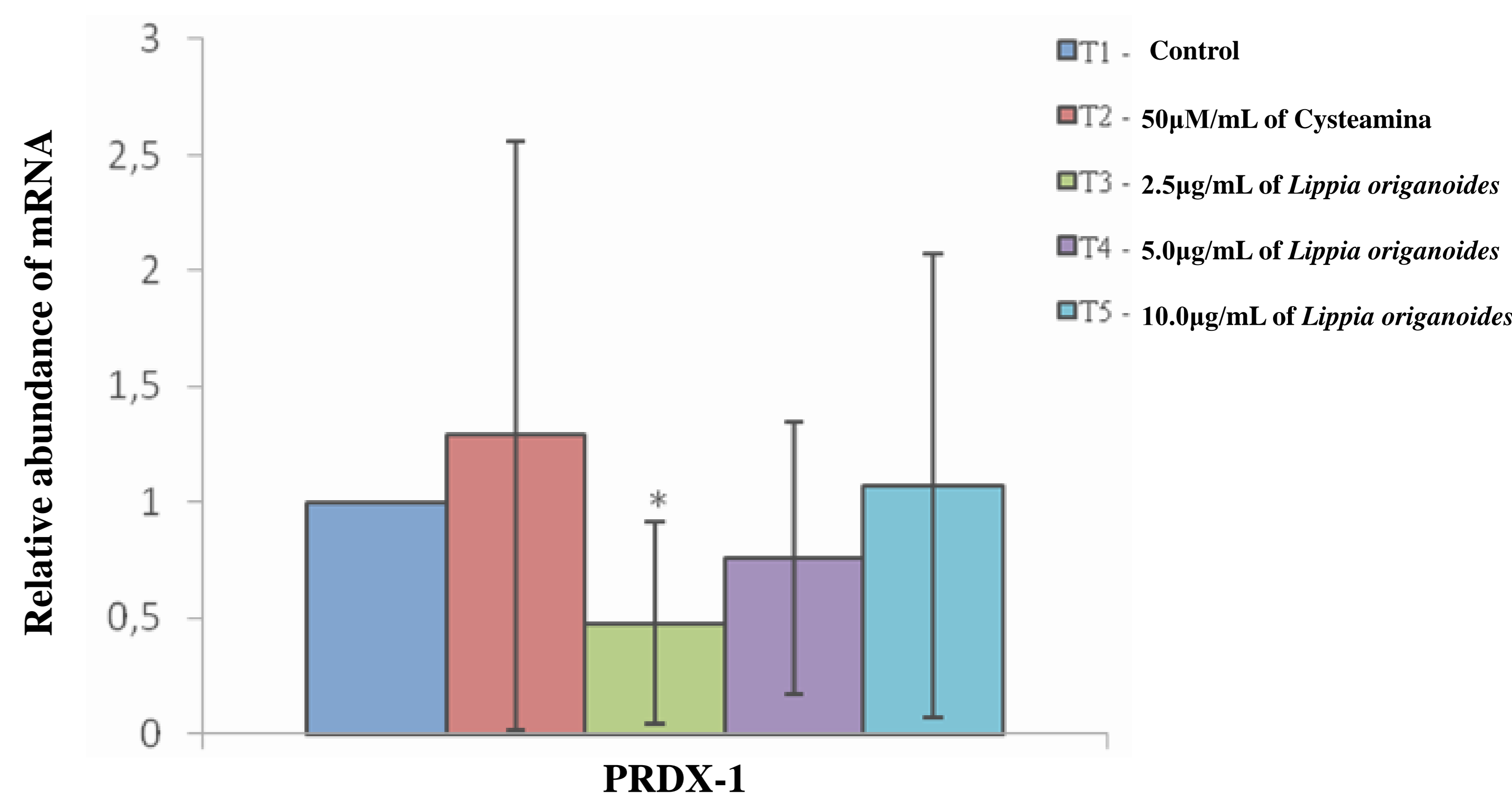
MATERIAL AND METHODS

Oocytes obtained from slaughterhouse ovaries were matured and fertilized *in vitro* according to standard procedure for bovine species. After 20 hours of fertilization, the possible zygotes were cultivated in SOFm medium supplemented with antioxidant, constituting five different treatments:



On the seventh day of culture (D7), blastocyst (B1) stage embryos were washed in DPBS added with 0.1% PVP and transferred to 1.5 mL RNA-free cryotubes in a volume of 5µL. Cryotubes were immediately immersed in liquid nitrogen (-196°C) and stored at -80°C until the moment of gene expression analysis by RT-PCR. The RNA samples were immediately reverse transcribed using the TaqMan® Reverse Transcription Reagents kit (Applied Biosystems®, Carlsbad, CA, USA) with Oligo dT, to obtain complementary DNA (cDNA) for the target gene PRDX-1.

RESULTS



The gene expression of PRDX-1 in bovine embryos produced *in vitro* did not differ in the amount of transcripts between the control group without supplementation, T2 (1.28 ± 1.27) containing antioxidant Cysteamina, T4 (0.75 ± 0.58) and T5 (1.07 ± 0.99) (P>0.05). However, embryonic culture medium containing a lower concentration of antioxidant obtained from essential oil of *Lippia origanoides* (2.5µg/mL; T3) had lower (P<0.05) expression of PRDX-1 (0.47 ± 0.43) which may be considered underexpressed in embryos cultured at these concentrations.

CONCLUSION

The present study demonstrated for the first time the low amount of PRDX transcripts in embryonic culture media supplemented with essential oil of *Lippia origanoides*, mainly at a concentration of 2.5µg/mL. These results suggest that the difference in the expression of this gene reflects in the intracellular situation, producing a lower protection against oxidative stress in embryos cultivated in this treatment.

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