ANIMAL REPRODUCTION

Official journal of the Brazilian College of Animal Reproduction

v.15, n.3 **July/September** **2018**

Contents

Proceedings of the 32nd Annual Meeting of the Brazilian Embryo Technology Society (SBTE); Florianópolis, SC, Brazil, August 16th to 18th, 2018, and 34th Annual Meeting of the European Embryo Transfer Association (AETE); Nantes, France, September 7th and 8th, 2018

Conferences papers

Conference abstracts

32nd Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

34rd Meeting of the Association of Embryo Transfer in Europe (AETE)

A095 OPU-IVP and ET

Ethanolic extracts of cerrado plants on the oxidative stress of *in vitro*-produced bovine embryos

A.A.G. Fidelis[1,2,3], F.R. Melo[1], L.O. Leme[3], T.S. Kawamoto[3], P.R. Adona[4], M.A.N. Dode[3]

[1]UniCeub - Centro Universitário de Brasília, Brasília, DF, Brasil; [2]UnB - Programa de Pós-Graduação em Ciências Animais - Universidade de Brasília, Brasília, DF, Brasil; [3]Embrapa Cenargen - Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brasil; [4]Unopar - Universidade do Norte do Paraná - Centro de Pesquisa e Pós-Graduação, Londrina, PR, Brasil.

The *in vitro* embryo culture induces an excessive production of reactive oxygen species (ROS) and affects blastocysts production. Medium supplementation with antioxidants agents in culture is an interesting alternative to minimize those effects. The present study evaluated the effect of ethanolic extracts obtained from cerrado plants on the oxidative stress of IVP embryos. Bovine ovaries from slaughterhouse were used to obtain grade I and II oocytes, which were submitted to maturation, fertilization (D0) and *in vitro* culture. Four groups were used in the culture: a control group subject to high oxygen tension (G20%), a group subject to low oxygen tension (G5%), besides two groups cultivated under high oxygen tension, supplemented with 0.01mg/mL of cagaite extract (GCag) and another group with 0.01mg/mL of murici extract (GMuri). Cleavage (D2) and blastocyst rates (D6 and D7) were evaluated. D7 expanded blastocysts were used to evaluate ROS, glutathione (GSH) and gene expression. ROS levels were analyzed by confocal microscopy using H2DCFDA (6-carboxy-20,70-dichlorodihydrofluorescein diacetate), and GSH on epifluorescence microscopy with Cell Tracker Blue (4-chloromethyl- 6,8-difluoro-7-hydroxyocoumarin). The transcripts level of genes involved in apoptosis (BAX, BCL21L, CASP3 and CASP8) and in the ROS metabolic pathways (SOD2, CAT, GPx4 and PRDX3) was determined by qPCR, with GAPDH used as a housekeeping gene. Data were analyzed by ANOVA and the means compared by Tukey test, at 5%. A total of 2135 oocytes were used and 893 embryos were produced (41.8%). Embryo production was similar (P>0.05) among G20% (cleavage: 87.6 ± 8.1%, D6: 26.5 ± 12.2% and D7: 42.7 ± 6.2%), GCag (89 ± 7.3%, 23.9 ± 10.3%, 43 ± 6.2%), GMuri (89 ± 9.5%, 26.7 ± 16% and 40.1 ± 8.4%) and G5% (88 ± 9.5, 26.7 ± 16, 40.1 ± 8.4). ROS and GSH emitted fluorescence were also similar (P> 0.05) among G20% (105.24 ± 26.04 and 156.36 ± 11.39), GCag (125, 92 ± 31.82 and 159.98 ± 10.89), GMuri (135.25 ± 29.05 and 155.36 ± 14.07) and G5% (116.05 ± 27.51 and 151.37 ± 17.45). The results showed that transcripts levels of apoptosis-related genes were similar (P>0.05) among the groups. However, genes involved in ROS metabolism were differentially expressed in the treatments. Abundance of GPX4 transcripts was higher (p <0.05) in the groups cultured with cagaite and murici than the G5% group, and PRDX3 transcripts was higher (p <0.05) in the GMuri group than the G5% group. The other transcripts analyzed (SOD2 and CAT) were similar among treatments (P>0.05). Supplementation of culture medium with extracts (0.01mg / mL) of cagaita and murici increased transcripts levels for genes related to antioxidant function (GPx4 and PRDX3), although it did not increased embryo production. Therefore, those extracts can be an alternative to reduce oxidative stress caused by IVP adverse conditions.