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Extracts from cerrado plants as antioxidant agents on *in vitro* embryo production

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The present study evaluated the effect of ethanolic extracts of plants from Cerrado- Brazil, containing high levels of polyphenols (antioxidant), on in vitro embryo production (IVEP) in cattle. Ovaries from slaughterhouse were used to collect grade I and II cumulus-oocyte-complexes (COC), which were submitted to in vitro maturation, fertilization (D0) and culture. Different concentrations (0; 1mg/mL; 01mg/mL and 0.01mg/mL) of cagaita (Eugenia dysenterica) and murici (Byirsonima crassifolia) extracts were added to the culture medium during embryo development. The parameters analyzed were: cleavage rate on D3, blastocyst rate in D6, D7 and total and apoptotic cells number by TUNEL method. The ability of those extracts to request free radicals from the culture medium was analyzed by ABTS colorimetric method. To do that an aliquot of the culture media was collected from each treatment drop at two different time points (D0 and D7). Data were analyzed by analysis of variance - ANOVA and the means were compared by TUKEY, with a significance level of 5%.The results of embryo production did not differ between the control group: cleavage 80.5% (136/169), blastocyst rate D6: 30.2% (51/169) and D7: 41% (69/169) and the groups treated with murici 0.1mg: 81.9% (149/182), 23.6% (43/182) and 35.2% (64/182) and 0.01mg: 78% (127/163), 32.% (52/163), 38.7 % (63/163). The total embryonic cells and the proportion of apoptotic cell in expanded blastocyst (BX) in D7 were similar among the groups (P=NS). Except for the 1mg group, that showed high toxicity and death already on cleavage stage evaluation. Regarding the capacity of polyphenols to remove free radical, no differences (P>0.05) between those same groups (control, 0.1 and 0.01mg). The only difference detected ($p < 0.05$) was also for the 1mg/ml group, which showed an increase on the amount of free radicals. However, the cagaita extract showed a similar behavior for cleavage rates in control group: 80.6% (179/222); 1mg: 78.3% (177/226); 0.1mg: 81% (187/231); 0.01mg: 82.5% (184/223). Yet, when evaluating the blastocyst rate at D6, a lower rate ($p<0.05$) was observed for the 1mg group 27/226 (12%) compared to the to control group 57/222 (25.7%), 0.1mg 59/231 (25.5%) and 0.01mg 76/223 (34%) groups. The same profile was observed at D7, with 45.5% of embryos in the control (101/222); 35% in the 1mg group (79/226 $p < 0.05$); 42% in 0.1mg (98/231) and 50% in the 0.01mg (112/223) groups. The number of BX cells was similar among all groups. However, the proportion of apoptotic cells was lower ($p < 0.01$) in the group with 0.01mg cagaita (2.8%) than the others (control: 8.33%, 1mg: 5% and 0.1mg: 5.4%). The ABTS results for cagaita were similar for all groups. The results showed that extracts of the tested plants were toxic at concentration of 1mg/mL in However, when they were diluted thousand times, it was possible to observe a decrease in apoptotic cells using 0.01 mg of cagaita extract (Eugenia dysenterica). This same dilution of the murici extract did not affected any of the evaluated parameters. It can be concluded, that the cagaita extract (0,01mg/mL) is an alternative to be use as a coadjuvant for the reduction of the oxidative stress induced by the adverse conditions of IVP.