Animal Reproduction

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MORPHOLOGICAL EVALUATION OF IN VITRO BOVINE EMBRYOS EXPOSED TO ISOFLURANE

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Resumo

Inhaled anesthesics are one of the most used drugs for inductions and in anesthesic maintenance. The concentration of Isoflurane used may change under different conditions. Similars concentrations used in anesthesia for asssited reproduction techniques (1,5%), were able to reduce blastocyst capacitations in mouse expossed during the in vitro fertilization process. The aim of this work was to evaluate the embryotoxic effects of isoflurane, in the embryonic phase of maternal-zygotic transition, on the morphological quality of bovine blastocysts produced in vitro. Oocytes were selected (grade 1 and 2) and matured in vitro in TCM199 5% SFB at 38.5°C at 5% CO2. After 22-24h, the oocytes were transferred to IVF plate with TALP medium and inseminated at a concentration of 1x106 sperm/mL, for 22-24 hours. After fertilization, zygotes were cultured in SOF medium, supplemented with fetal bovine serum and BSA, in 5%CO2/90N2/5%O2. On the third day of culture, the treatment groups were exposed to isoflurane for 1, 3, and 6 hours (G1h, G3h, and G6h, respectively); and the control group (CG) was not subjected to anesthesia. The exposure was in a modular incubator (Phorma, 2L), 38.5°C, in 5%CO2/90N2/5%O2. This chamber was connected to an anesthesia machine (Hipnos - RWR) adjusted to provide a total airflow rate of 6 L/min, and vaporize concentrations of 3% isoflurane (based on the method CHETKOWSKI, 1988). On day 7, the groups were evaluated for blastocyst rate and morphological quality according to IETS: Q1 - excellent or good with up to 15% extruded cells; Q2 - fair, between 15 and 50% extruded cells; Q3 - poor, more than 75% extruded cells; Q4 - incompatible embryonic development, less than 25% viable embryonic mass. On day 10 the hatching rate was evaluated. The test of homogeneity of proportions was used to compare the variables of blastocysts; hatching and embryonic qualities. The results obtained indicated that the blastocyst rate in CG (45.26%; p-value < 0.001) was higher when compared to the exposed groups G1h (8.05%), G3h (7.33%) and G6h (5.15%). Significantly higher rates of hatched embryos were obtained in CG (Be: 83.81%; p-value < 0.001), when compared to the other treated groups, while among the exposed groups there was no significant difference in hatching (42.11%, p=0.405; 29.41%, p=0.196; 8.33% p=0.102, respectively G1, G3, G6). In the evaluation of embryo quality, there was a significant difference (p-value < 0.001), comparing the CG (Q1: 48.57%; Q2: 37.14%) with the other exposed groups G1h (Q1: 31.58%; Q2: 36.84%) G3h (Q1: 17.65%; Q2: 35.29%) G6h (Q1: 8.33%; Q2: 8.33%), observing superior embryo quality in the CG. Based on the results obtained, we conclude that the groups that were exposed to isoflurane obtained embryos with inferior morphological quality time-dependently. Exposure to isoflurane in the embryonic genome activation stages directly interfered with blastocyst production, hatchability and the quality of the resulting blastocysts.