Proceedings of the 28th Annual Meeting of the Brazilian Embryo Technology Society (SBTE), August 14 to 17th, 2014, Natal, RN, Brazil. Abstracts.

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Apoptosis in embryos exposed to the HSP90 inhibitor during *in vitro* maturation of bovine oocytes

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Keywords: 17AAG, heat shock, *in vitro* maturation.

The heat shock protein 90kda (HSP90) is a cytoprotective chaperone and its inhibition with 17-(allylamino)-17-demethoxygeldanamycin (17AAG, Sigma, St. Louis, USA) during IVM reduces oocyte competence, decreasing embryo production rate (Souza et al., 2013. Anim Reprod, 10:515). To assess possible damage in the embryos after exposure to different 17AAG concentrations during IVM, this study aimed to determine the total number of embryonic cells, apoptotic index of the inner cell mass (ICM) and the trophoblast (TE) of blastocysts. Immature oocytes aspirated from ovaries obtained from slaughterhouse were selected and randomly allocated in a factorial experiment design with three 17AAG concentrations (0, 1 and 2μM) and two-exposure time (12 and 24h) during IVM at 38.5°C under 5% CO2 and saturated humidity. Oocytes were *in vitro* fertilized (IVF) for 20h and incubated under the same maturation conditions. After IVF, the presumptive zygotes were denuded in a solution of PBS plus 0.1% hialuronidase and then cultured in CR2aa medium supplemented with 2.5% FCS (Nutricell, Campinas, Brazil) in a incubator at 38.5°C under 5% CO2, 5% O2 and 90% N2, and saturated humidity for 8 days. Cleavage was evaluated at day three and blastocysts were evaluated at day seven and day eight post-fertilization. Expanded blastocysts with 192h post-fertilizationfrom different treatments (0μM=42; 1μM=47; 2μM=39 and 12h=60; 24h=68) were fixed in 4% paraformaldehyde and available by TUNEL assay (DeadEndTMFlorimetric TUNEL System-Promega). Data from each treatment were analyzed by Generalized Linear Model procedure of SAS software (version 9.1) considering effect of exposure time, 17AAG concentration and interaction, and means were compared by Student Newman Keuls test. Values are shown as mean±SEM. There was no difference in the total number of cells, number of apoptotic cells and apoptotic index of cells analyzed embryos derived from treatments with different concentrations or time of exposure to the inhibitor, nor interaction between concentration and exposure time. However, there was a decrease in the number of cells of the ICM of embryos from oocytes treated with 1μM and 2μM of 17AAG compared to 0μM (36.36±1.26, 39.68±1.68 and 43.42±1.95, respectively). The data also showed differences (P <0.05) in the number of apoptotic cells of TE for 0μM, 1μM and 2μM (15.97±2.8a, 12.05±1.54a,b and 7.23±0.84b, respectively) and in the TE apoptotic index among embryos from oocytes exposed to a higher concentration of the 17AAG in maturation medium (2μM - 8.81±0.80) compared to the concentrations of 0μM and 1μM (15.51±1.78, 13.64±1.56, respectively). The use of 17AAG during IVM does not interfere on blastocyst total cell number or general apoptotic index, but it can reduce ICM cell number and TE apoptotic index, resulting in lower embryo developmental ability, as already reported in previous study.

Financial support: CNPq, FAPEMIG and FAPES.