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Effect of recombinant human FSH during *in vitro* maturation on apoptose of bovine blastocysts

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Previous study reported that recombinant human FSH (rhFSH) during in vitro maturation (IVM) allows blastocyst production at the same rate of FSH from porcine pituitary (Souza et al., 2012. Anim Reprod, 9:550). The present study aimed to evaluate the total cell number and cells in apoptosis in blastocysts generated from oocytes matured in vitro with different rhFSH concentrations. rhFSH was gently donated by Galactous Biotech Ltda (Concepción, Chile). Immature COCs obtained from slaughtered animals were randomly allocated in six treatments of *in vitro* maturation as follow: T1 - control with porcine FSH (pFSH - Pluset, Callier, Spain), T2 – without FSH; T3 – 0.0105 UI rhFSH; T4 – 0.021 UI rhFSH; T5 - 0.042 UI rhFSH and T6 – 0.084 UI rhFSH. IVM was performed in TCM199 medium (Invitrogen, Carlsbarg, USA) supplemented with 10% estrus cow serum for 24h at 38.5 ºC under 5% de CO_2 and 95% humidity. After IVM, oocytes were *in vitro* fertilized and cultured in modified CR2aa medium supplemented with 10% fetal calf serum (Nutricell, Campinas, Brasil) at 38.5 ºC under 5% de CO_2 and 95% humidity. Two hundred two blastocysts, from the different treatments, were fixed in 4% paraformaldehyde at the eighth day post-fertilization and evaluated by TUNEL technique for quantification of cells number and apoptotic index. Evaluation of total cell number and apoptotic index of inner cell mass (ICM) and trophoblast (TE) were also performed in some blastocysts (n=45). Localization of ICM and TE nuclei was based on their position in the captured images using ImageJ software. Data was submitted to analysis of variance and means compared by Student Newman Keul's test. Values are shown as mean ± SEM. There was no difference ($P>0.05$) on total cell number, total apoptotic cell number and apoptotic index in blastocysts from different treatments. When only TE was evaluated, both the total number of cells and the apoptotic index also were not different between treatments. However, in ICM, no difference in total number of cells was observed, but the blastocysts from T1 and T2 showed lower ($P<0.05$) apoptotic index (28.8±5.8 and 29.4±3.9%, respectively) than blastocysts from T3 and T4 (57.8±8.3 and 58.6±7.3%, respectively) but similar to T5 and T6 (44.8±4.4 and 46.3±3.8%, respectively). In all treatments the index of apoptotic cells in ICM (44.6±2.6) was higher than in TE (10.6±0.9). Overall, rhFSH during IVM does not influence the cells number or the index apoptotic in bovine blastocysts, nevertheless, can increase the apoptotic rate of ICM.

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