A155 OPU-IVP and ET

Effect of *in vitro* spom maturation system use on bovine embryos lipid score

G.R. Leal[1], C.A.S. Monteiro[2], H.F.R.A. Saraiva[1], P.M.S. Rosa[2], A.J.R. Camargo[3], A.L.R. Rodrigues[1], C.O.P. Vasconcelos[1], L.A.G. Nogueira[1], C.S. Oliveira[4], R.V. Serapião[3]

[1]Universidade Federal Fluminense; [2]EMBRAPA Gado de Leite; [3]PESAGRO-RIO; [4]EMBRAPA Gado de Leite-LRA-CESM.

Keywords: bovine embryo, forskolin, Lipidic score.

Oocyte quality determines the proportion of oocytes that will develop to blastocyst stage, and although the lipid content is important in oocyte development, the elevated number of lipid droplets have been associated with reduced cryosurvival, which is a relevant issue for embryo IVP system. The *in vitro* maturation system (IVM) Simulated Physiological Oocyte Maturation (SPOM) mimics the physiological maturation events by using AMPc modulators that promote the increase of oocyte competence. Forskolin is an example of AMPc modulator and this molecule has a lipolytic action. The aim of this study was to evaluate the effect of the SPOM system (Albuz, Hum. Reprod, v25, p12; 2010) on embryonic lipid score (relation between the lipid content and the total number of cells,TNC). In four replicates oocytes were obtained from slaughterhouse ovaries, selected and randomly divided into three groups: SPOM, CONTROL 1 (C1) e CONTROL 2 (C2). The MIV occurred during 24 h in C1 (TCM199 medium without FBS) and C2 (commercial medium Bioklone® Animal Reproduction, Sao Paulo, Brazil/ with FBS) in culture incubator at 38.5° C, 5% CO_2 in atmospheric air and high humidity. In SPOM group, oocytes were incubated in pre-IVM (TCM 199 medium with 100µM Forskolin and 500µM IBMX) for 2 h followed by an extended IVM (TCM 199 medium + 20µM cilostamide) period (28 h) under the same conditions as described for other groups. After IVM, oocytes were fertilized, and transfered to culture droplets, where they remained for seven (n=25-46 per group) or 9 (n=6-9 per group) days. The lipid content analysis and TNC meansure were performed using Oil Red and HOECHST 33342 staining, respectively. The lipid score was obtained by the stained lipid area divided by the TNC of each embryo and the averages were compared according to the days (D7 or D9), for each treatment, by the Kruskal-Wallis test in the Instat GraphPad program, with significance level of 5%. There was no difference ($P<0.05$) between groups (SPOM: 298.5 ± 139.9a ; C1: 226.0 ± 75.7; C2: 211.3 ± 1003a) in D7, suggesting that the time of exposure to Forskolin was not enough to ensure lipolytic action. At D9, only the C2 showed increase compared to others (SPOM: 154.0 ± 27.1a; C1: 135.7 ± 26.2a ; C2: 291.8 ± 71.4b); possibly due to the FBS effect on lipid accumulation. Between D7 and D9, there was a reduction ($P<0.05$) in the lipid score at SPOM (298.5± 139.9 vs 154.0± 27.1) and C1 (226.0 ±75.7 vs 135.7± 26.2) groups, which can be explained by the increase of embryonic metabolic consumption with the advance of embryonic development. However, the C2 showed no difference ($P>0.05$) between day 7 and 9 (211.3 ± 100.3 vs 291.8 ± 71.4), suggesting that the FCS effect on lipid accumulation was greater than the embryos metabolic activity. It was concluded that SPOM system had no effect in lipid score of *in vitro* produced embryos.

Financial support: FAPERJ(E26/111.155/2013), FAPEMIG and CAPES.