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Effect of camp modulators on *in vitro* pre maturation in production rate and lipid content of crossbred bovine embryos

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Simulated Physiological Oocyte Maturation system (SPOM; Albuz, Hum Reprod, vol 25, p 12, 2010) was developed to improve the oocytes quality by using cAMP modulators, among which forskolin, which also has delipidant action - desirable for structures directed for cryopreservation. In this system, two stages are proposed: Pre-IVM and extended IVM. The aim of this study was to evaluate the effects of only the pre-IVM step (2h culture in the presence of forskolin and IBMX) on blastocyst rates and lipid content of *in vitro* produced bovine embryos. COCs obtained from slaughterhouse ovaries in three replicates, were selected based on the number of cumulus cells and homogeneous cytoplasm and randomly distributed into two groups: control [C, n = 84; Standard IVM for 24 hours in the commercial medium (Bioklone® Animal Reproduction, Brazil)] and pre-IVM [PM, n = 99; pre-IVM for 2 hours in pre-IVM medium (TCM 199-Hepes, BSA 1,6mg/mL, sodium pyruvate 100mM, ITS 100x, penicillin 10.000UI, streptomycin 10mg/mL, forskolin 100μM and IBMX 500μM) followed by standard IVM]. After IVM, the groups underwent at the same time IVF in TALP and IVC in SOF, both using Bioklone® medium. The cleavage and blastocyst rates were evaluated in D3 and D7, respectively. The blastocysts obtained were fixed in 4% PFA in D7 and stored at 4 ° C until subjected to Oil Red staining technique for lipid content evaluation by analyzing stained area fraction using ImageJ software. The cleavage and blastocyst rates were compared using Fisher's exact test (different averages identified with distinct superscript letters), and the mean lipid stained area fraction compared by Mann Whitney test. Statistical analyzes were performed using GraphPad INSTAT, at a 5% significance level. There was no difference (P>0.05) between the cleavage rates of C and PM (80.95%a - 68 vs 82.83%a - 82), blastocyst rate/total oocytes (39.28%a - 33 vs 28.28%a - 28) and blastocyst rate/cleaved (p = 0.0951; 48.53%a vs 34.14%a) respectively. The mean stained area fraction for lipid to the C was 30.60 ± 5.48 vs 40.23 ± 4.01 for the PM (p>0.05). These results suggest that isolated from SPOM system, usage of pre-IVM step before comercial IVM resulted in no improvement in blastocyst rates, nor the delipidant effect of forskolin was observed in resulting blastocysts.

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