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laboratory. Then, oocytes were obtained by scraping follicles. We first analyzed the effect of maintaining oocytes in holding medium overnight previous to maturation. Second, two maturation media were compared, TCM-199 vs DMEM-F12, both supplemented with EGF 0,1mg/mL, 10% FCS, 8 UI/mL FSH, 2.5 µg/mL LH and 0.5% gentamicin, cultured in microdroplets at 38.2°C in 5% CO2 in air and maximum humidity for 26-28 h. Then, we studied the capacity of fresh, refrigerated and frozen sperm to activate murine oocytes after ICSI and resume meiosis. Afterwards, we compared two culture media (DMEM-F12 vs SOF, both supplemented with 10% FCS and 0.5% gentamicin) cultured in 5 µL microdroplets at 38.2°C in 5% CO2 in air and maximum humidity. Finally, we evaluated embryo quality by standard morphology grading and blastocyst rate.

Holding medium overnight previous to maturation improved equine oocyte maturation rate compared to perform maturation directly after the obtainment of oocytes (85.9 ± 3.1a vs 54.2 ± 27.03b respectively). A higher percentage of matured oocytes was obtained using DMEM-F12 compared to TCM-199 (81.4 ± 23.3a vs 50 ± 0.0b respectively). Also, a higher percentage of cleavage (68.4 ± 15.1 vs 56.3 ± 7.2) and blastocyst (32.3 ± 5.2a vs 18.8 ± 12.5b) rates were obtained using DMEM-F12 compared to SOF media. DMEM-F12 medium produced also better morphology than those embryos cultured in SOF. We obtained a higher oocyte activation using both refrigerated or frozen sperm (48.9 ± 21.5 % and 59.1 ± 20.1 % respectively) compared to fresh sperm (31.4 ± 23.4 %). In conclusion, our optimized protocol includes the use of holding medium previous to maturation, the use of refrigerated or frozen sperm, and the use of DMEM-F12 for both maturation and culture.

PW1256 - Effect of cAMP modulators during oocyte in vitro maturation on apoptosis rate of bovine embryos

Authors and co-authors:

Pasolini Renata (1), Graciosa Maria Alice (1), Leal Gabriela (2), Monteiro Clara Ana (2), Oliveira Clara (3), Camargo Agostinho (1), Serapião Raquel (1)

(1) PESAGRO-Rio, Animal Reproduction, 24120191, Niteroi, Brazil

(2) Universidade Federal Fluminense, Veterinary Clinic and Pathology, 24320340, Niteroi, Brazil

(3) Embrapa Dairy Cattle, LRA CESM, 27640000, Valença, Brazil

The brazilian economic growth is due in part to agricultural sector evolution in recent decades. In vitro production (IVP) of cattle embryos represents an important tool for the dairy production systems, mainly for increasing the reproductive efficiency of genetically superior animals. The oocyte quality is considered the key factor that influences IVP and 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 (Albuz, Hum. Reprod,v25, p12; 2010). The aim of this study was to evaluate the effect of SPOM system on bovine embryos quality using the total number of cells (TNC) and apoptosis rate as parameters for it. Four replications were held in which oocytes were obtained from slaughterhouse ovaries, selected and randomly divided into two groups: CONTROL(C) and SPOM (S). The IVM lasted 24 hours for group C (TCM 199 medium without FBS) in culture oven at 38.5°C, 5% CO2 in atmospheric air and high humidity. In SPOM system, oocytes were, for 2h, in pre-IVM (TCM 199 medium + 100µM Forskolin + 500µM IBMX) and followed for extended IVM (conventional medium + 20µM cilostamide) for 28h under the same conditions as control group. After IVM, oocytes were fertilized with semen from a single holstein bull, and transfered to culture droplets, where they remained for 7 days. The TNC analysis was measured after staining with HOECHST 33342 and results were analyzed by Student's t test. To analyze the apoptosis rate, it was used Caspase 3 immunofluorescence reaction, and the results were compared by Fisher exact test. All statistical analyzes were performed in Instat GraphPad program, with a 5% significance level. There was no significant difference (p> 0.05) between groups (S:n=9/ C:n=10) on TNC (S: 108.78 ± 18.2 a/ C: 103.5 ± 31.7 a) and on apoptosis rate (S: 2,86 % a/ C: 2,80% a). Some studies showed beneficial effect on embryos quality using this system, however, our results demonstrated no effect on evaluated parameters. We believe that, although TNC and apoptosis rate are important to access embryo´s quality, further studies are necessary to investigate the effect of the system in others quality parameters.

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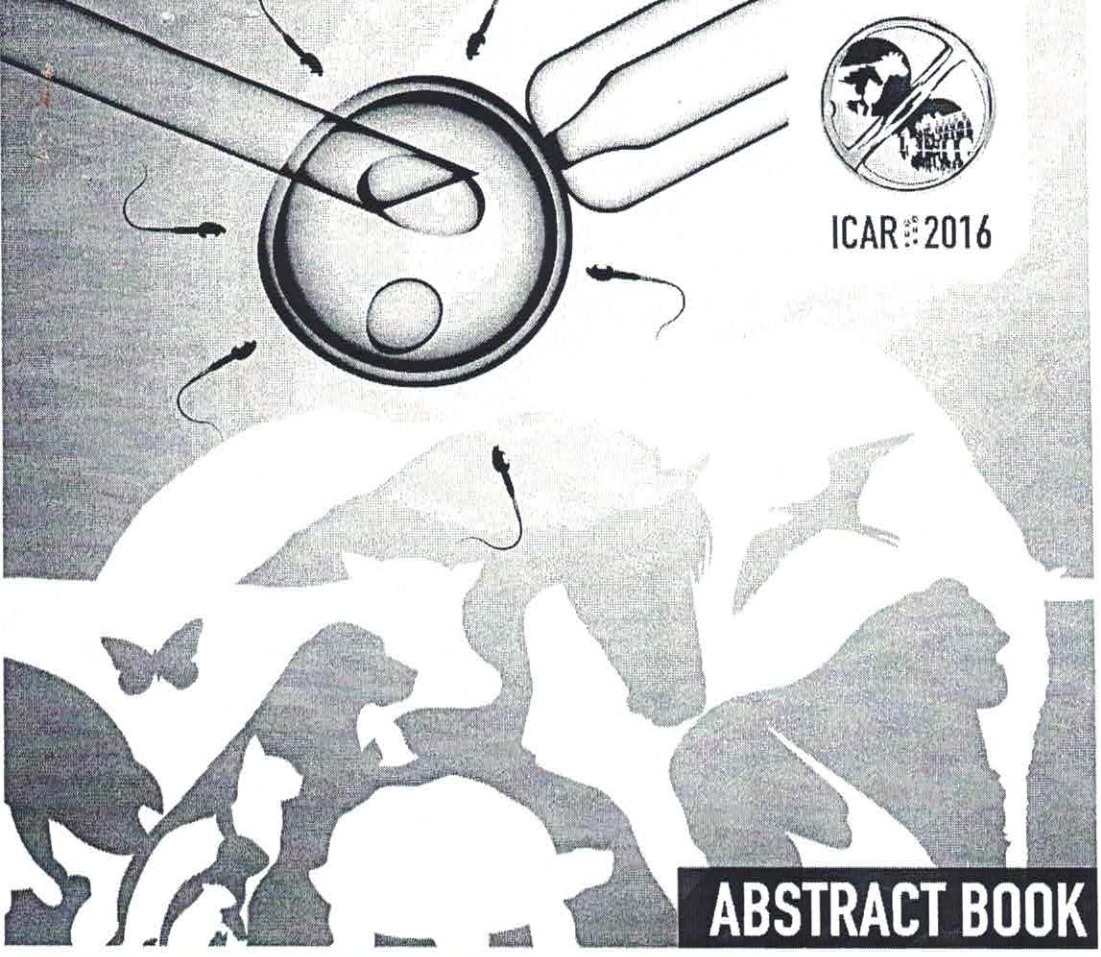

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ABSTRACT BOOK

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