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A304 Cryopreservation and cryobiology, diagnosis through imaging, molecular biology and "omics"

Effect of cAMP modulators during oocyte *in vitro* maturation on nuclear maturation and cytoskeleton integrity of vitrified bovine oocytes

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Cryopreservation of oocytes is a strategic tool in embryo IVP but with limited use due to the complex cellular structure of oocytes, being oocyte quality a factor that influences the success of the technique. In view of the role of IVM on oocyte quality, Simulated Physiological System Oocyte Maturation (SPOM; Albuz, Hum Reprod, vol 25, p 12; 2010), which utilizes cAMP modulators to achieve greater oocyte competence by the extension of meiosis block, was developed. The aim of this study was to investigate the effect of SPOM system on nuclear maturation and cytoskeletal integrity of vitrified bovine oocytes. Oocytes from slaughterhouse ovaries were divided into 8 groups: G1 (immature oocytes); G2 (matured in standard medium without FCS / 24 h); (G3 subjected to pre-IVM / 2 h in the presence of modulators of cAMP, Forskolin (100μM) and IBMX (500μM), and then the extended IVM / 28 h with Cilostamide (20μM) and FSH); G4 (immature oocytes vitrified and subjected to conventional IVM); G5 (immature vitrified and subjected to pre-IVM and extended IVM); G6 (submitted to pre-IVM, vitrified, and then the extended IVM); G7 (matured in commercial IVM - Bioklone ® Animal Reproduction, São Paulo, Brazil - with SFB / 24 h); G8 (immature vitrified and subject to commercial IVM, with SFB). For analysis of nuclear maturation, oocytes (n = 429 obtained in 3 replicates, 15 to 75 per experimental group) were stained with Hoechst 33324 for obtaining the rate of matured oocytes (MII). Cytoskeletal actin filaments were stained with Phalloidin Atto-532 to evaluate the actin staining patterns (Stained / Uninjured or not stained / Injured; n = 373 obtained in 2 replicates, 9 to 73 per experimental group). The M II rate was evaluated by chi-square test ($\chi 2$) and the percentage of staining patterns/integrity of actin by Fisher's exact test, in Instat GraphPad program, the significance level of 5%. The MII rate observed in groups was: G1 (Immature): 6.67 ^{a}; G2 (Standard IVM): 77.78 ^{c}; G3 (SPOM IVM): 76.19 ^{c}; G4 (VIT/Standard IVM): 31.43 ^{ab}; G5 (VIT/pré-IVM/extended IVM): 18.57 ^{a}; G6 (pre-IVM/VIT/extended IVM):): 39.47 ^{b}; G7 (Commercial IVM): 71,74 ^{c}; G8 (VIT/Commercial IVM): 25.71 ^{ab}. This result suggests that in vitrified groups maturation was impaired, but to a lesser extent in the group undergoing pre-MIV before vitrification. Regarding the pattern of actin (Uninjured / Injured), we observed: G1: 100.0^{a} / 0.0; G2: 100.0^{a} / 0.0; G3: 100.0^{a}/0.0; G4: 50.0^{bd} / 50.0; G5: 26.0 ^{c} / 74.; G6:60.3 ^{b} / 39.7; G7: 94,2^{a} / 5.8; G8: 37.9 ^{cd} / 62.1. Therefore, our results suggest that in vitrified groups the injured integrity pattern is predominant, except in the group undergoing pre-MIV before vitrification. The pre-IVM SPOM system favors meiotic progression and cytoskeletal integrity of oocytes undergoing vitrification, and its use can improve rates at oocyte cryopreservation programs.

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