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Development and quality of embryos generated by zinc chelation of bovine eggs

Juan Camera [1], Juan Pedro Cabeza [3], Minerva Yauri Felipe [2,3], Olinda Briski [2,3], Daniel Salamone [2,3], Andrés Gambini [1,3,2]

[1] PEqFAUBA - Producción Equina, Facultad de Agronomía, UBA (Av. San Martín 4453, CABA, Argentina), [2] CONICET - Consejo Nacional de Investigaciones Científicas y Técnicas (Godoy Cruz 2290, CABA, Argentina), [3] LABBA - Laboratorio de Biotecnología Animal, Facultad de Agronomía, UBA (Av. San Martín 4453, CABA, Argentina)

Resumo

Oocyte activation is considered one of the most crucial steps for successful embryo development. Although naturally triggered by sperm, oocyte activation can be achieved by artificial means to improve the development of fertilized eggs or to produce nuclear transfer embryos. After sperm-egg fusion, intracytoplasmic rises of calcium (Ca) induce the release of zinc (Zn) out of the egg (Zn sparks). Both phenomenons are known to play an essential role in the oocyte activation process. Our work aimed to determine the optimal condition for inducing activation of bovine eggs using the novel Zn chelator 1,10-phenanthroline (PHEN, Sigma P9375) and to compare the parthenogenetic developmental rates and embryo quality with IVF and -Ionomycin- induced (IONO) embryos. In Experiment 1, we compared SOF and TALP-H media for Zn chelation at an established condition (0.5mM for 1h; Uh et al., Theriogenology, 125:259-267, 2019). In Experiment 2, we compared incubation conditions to the Zn chelator for optimising the activation protocol. Embryo quality was assessed by immunofluorescence (IF) of SOX2, SOX17 and CDX2. Oocyte collection, IVM and IVF procedures were performed as reported (Ynsaurralde et al., Theriogenology, 148:140-148, 2020). Eggs were activated using PHEN or 5mM of IONO for 4m in TALP-H. After treatment, zygotes were incubated 3h in 1.9mM of 6-Dimethylaminopurine and cultured in SOF media. Fisher's exact test was performed for statistical analysis. Day 7 blastocysts were fixed and subjected to IF using SOX2, SOX17 and CDX2 antibodies followed by statistical analysis as reported by Gambini et al., PLOS ONE 15(9):e0238948, 2020. In Experiment 1, PHEN-TALP-H resulted in higher cleavage rates compared to PHEN-SOF and was used for experiment 2 (IONO, n=80, 95.00%; PHEN-SOF, n=73, 61.64%; PHEN-TALP-H, n=93, 76.34%). PHEN blastocyst rates were significantly lower compared to the control (IONO, 76,25%; PHEN-SOF, 3,26%; PHEN-TALP-H, 15,05%). In Experiment 2, PHEN developmental rates were lower than embryos with artificial (IONO) or sperm-induced (IVF) activation (IVF, n=101, 41.58%; IONO, n=83 50.60%; PHEN 0.5mM for 30m, n=73 20.55%; PHEN 0.5mM for 1h, n=82, 28.04%; PHEN 1mM for 30m, n=72, 27.78%; PHEN 1mM for 1h, n=72, 19.44). Blastocyst produced with PHEN 0.5mM for 1h showed a significantly less total cell number compared to IVF (mean±SEM, IVF 116.5±7.56; IONO, 91.00±7.30; PHEN 85.19±5.16). Moreover, PHEN blastocysts showed a higher number of SOX2+ cells (52.70±4.5) than IVF (29.80±4.5), but not with IONO (43.03±6.68). Interestingly, more than 40% of the PHEN embryos showed a scattered pattern of SOX2 expression compared with less than 15% in IONO and IVF groups. Our observations suggest that even though blastocyst development can be achieved in vitro using a Zn chelator in bovine bypassing Ca oscillations, developmental rates and blastocyst quality are compromised compared to embryos generated with artificial or sperm-induced calcium oscillations.