352 DISSOCIATION OF NUCLEAR AND CYTOPLASMIC MATURATION OF BOVINE OOCYTES IN DEFINED CULTURE SYSTEM

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Abstract

Insufficient cytoplasmic maturation of MII oocytes may be one reason of the low rate of embryo production *in vitro*. To solve this problem, a two-step procedure of IVM is tested. In this case, there is a prematuration period (PMP) in which meiosis is arrested, followed by a resumption period (RP) during which the oocytes are liberated from the inhibition of meiosis. Preliminary studies have shown that a defined medium (DM) inhibits nuclear oocyte maturation (NM) (Unpublished data). Thus, our purpose was to verify if meiosis arrest is followed by an inhibition of cytoplasmic maturation (CM). Herein we investigated the effects of DM on NM and CM using the two-step procedure for IVM. COCs were selected from bovine ovaries and cultured in non-defined medium (NDM), composed of TCM-199 suplemented with 10% fetal calf serum and 200 ng mL^{-1} FSH or in a defined medium (DM), MEMα supplemented with 0.1% Polyvinyl Alcohol. Both media are from Invitrogen-Gibco/BRL, Grand Island, NY, USA. In the PMP (experiment 1), COCs (n = 232) were distributed in G1 (NDM) or G2 (DM) and cultured for 24 h as usual. After this, NM and CM were analyzed. As inhibition of meiosis was observed in G2, a second step of culture (experiment 2) was performed to evaluate the ability of the COCs to reverse meiosis arrest. Thus, COCs (n =202) were distributed in G1 (NDM) or G3 (DM +NDM). COCs from G1 were cultivated in NDM for 24 h, and that ones from G3 in DM for 24 h followed by cultivation in NDM for an additional 24 h (RP). After this, NM and CM were analyzed. The CM evaluation (distribution of organelles) was performed in fresh oocytes (0 h) and in G1, G2, and G3 after usual preparation for transmission electron microscopy (TEM) analysis. The NM data were analyzed by Chi-square test. After the PMP period, G1 COCs presented 83.17% (84/101) of complete nuclear maturation whereas G2 presented 74.05% (97/131) of NM inhibition (oocytes in GV or GVBD) ($P < 0.05$). This meiosis arrest observed in DM was reversed after 24 h of cultivation in NDM, 79.17% (90/110) of G3 COCs were at MII. The analysis of CM has shown fresh COCs with ultrastructural characteristics of immaturity, as presence of mitochondria in the periphery of the ooplasm, and cortical granule clusters in the ooplasm; G1 COCs with mature cytoplasmic characteristics as mitochondria spread throughout the ooplasm and cortical granules located close to the oolema and G2 COCs with both immature and mature characteristics as presence of ooplasm granule clusters and presence of mitochondria spread throughout the ooplasm. G3 have shown mature cytoplasmic characteristics in the end of RP. In conclusion, a two-step procedure of IVM using a DM was able to maintain meiosis arrest while the cytoplasm undergoes maturation (G2). This could be a strategy to improve the rate of viable embryos obtained at *in vitro* embryo production.

FAP-DF, Finatec, CNPq, Capes.

Reproduction, Fertility and Development 22(1) 332–332 doi:10.1071/RDv22n1Ab352
Published: 08 December 2009

Reproduction, Fertility and Development

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Volume 22 Number 1 2010
Proceedings of the Annual Conference of the International Embryo Transfer Society, Córdoba, Argentina, 9–12 January 2010
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