245 EFFECT OF BOVINE OOCYTE MATURATION SYSTEM ON RELATIVE ABUNDANCE OF mRNA

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Abstract

The oocyte cytoplasm contains several transcripts that are important for early pre-implantation embryo development, and alterations on the amount of these stored mRNA can disturb oocyte competence. The aim of this study was to evaluate the relative abundance of specific transcripts in oocytes matured *in vivo* or *in vitro*. For *in vitro* maturation, immature oocytes were obtained by ovum pickup from 4 crossbred cows (group 1: G1) or from ovaries collected at a slaughterhouse (group 2: G2) and matured in TCM-199 containing 10% estrus cow serum and 2 µg FSH for 24 h under 5% CO^2 in air at 38.5°C. For *in vivo* maturation, the same crossbred cows used in G1 received a progesterone intravaginal implant (CIDR®, Eazi-Breed CIDRO, São Paulo, Brazil) and 2 mg of estradiol benzoate (Estrogin®, Farmavet, São Paulo, Brazil) on Day 0. On Day 4, cows were superstimulated with 180 mg FSH (Folltropin®, Bioniche, Canada) injected in 6 decreasing doses every 12 h, and on Day 6, the cows received 0.53 mg of sodium cloprostenol (Sincrocio®, Cooper, São Paulo, Brazil). On Day 7, CIDR® was removed and 2.5 mg of gonadorelina (Gestran-Plus®, Tecnopec, São Paulo, Brazil) was injected. Ovum pickup was performed 18 h after gonadorelina injection. Oocytes with expanded cumulus cell were then pooled and used as *in vivo*-matured oocytes (group 3: G3). Oocytes from all groups were denuded and frozen in liquid nitrogen. Pools of 10 oocytes for each group were subject to RNA extraction and reverse transcription. cDNA was amplified by real-time PCR using the beta-actin gene as the endogenous reference. The transcripts analyzed are encoded by TEA domain 2 (*TEAD2*), high mobility group N1 (*HMGN*), zygotic arrest 1 (*ZAR1*), maternal antigen that embryo requires (*MATER*), growth differentiation factor-9 (*GDF9*), and peroxiredoxin 1 (*PRDX1*) genes. Results were analyzed by REST software v.2 using the pair-wise, fixed reallocation randomization test. Data from G3 were used as calibrator. There was no difference ($P > 0.05$) on relative abundance of all transcripts between pools of oocytes matured *in vitro* or *in vivo* obtained from the same cows (G1 and G3, respectively). However, the relative abundance of *GDF9* (0.22 ± 0.04-fold) was less ($P < 0.05$), whereas the relative abundance of *TEAD2* transcripts (4.27 ± 2.14-fold) was greater ($P < 0.05$) for *in vitro*-matured oocytes obtained from slaugterhouse ovaries (G2) when compared with *in vivo*-matured oocytes (G3). No difference ($P > 0.05$) on relative abundance was found between G2 and G3 for the other genes. These data suggest that *in vitro* maturation does not alter the relative abundance of some transcripts stored into oocytes when compared with the ones stored in oocytes obtained from the same donors by means of multiple ovulation.

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