Functional activity of actin cytoskeleton in prolong-cultured bovine oocytes

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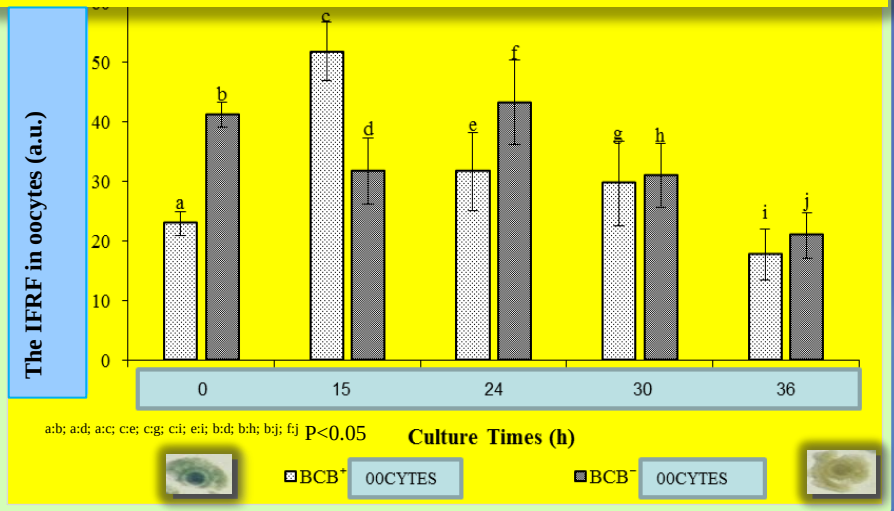
INTRODUCTION. Actin cytoskeleton is involved in oocyte maturation and competence acquisition. **THE AIM** of study was to evaluate the kinetic of the functional activity of actin cytoskeleton [the intensity of fluorescence of rhodamine-phalloidin (IFRF, a.u.) conjugated with actin filaments] in oocytes depending on the functional status and culture time. Brilliant cresyl blue (BCB) staining has been used for evaluation of the functional status of oocyte [growing (BCB^-) or fully grown oocytes (BCB^+),

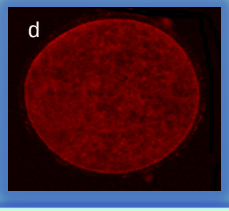
Fig. The IFRF in growing (BCB^-) or fully grown oocytes (BCB^+) during IVM (n oocytes - 324).

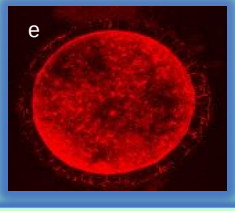


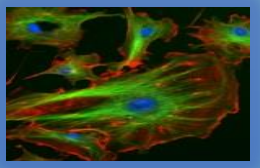
MATERIALS & METHODS. Oocytes were cultured 36 h in TCM 199 + 10% (v/v) FCS + 50 ng/ml PRL with granulosa 10^6 cells /ml. For assessment of chromatin and IFRF oocytes were incubated in rhodamine-phalloidin (RF, R415 Invitrogen, Moscow, Russia), 1 IU/ml, for 30 min to label actin. Then oocytes were incubated in 4',6-diamidino-2-phenylindole, 10 |µg/ml, for 10 min to label chromatin. Oocytes were examined using confocal laser scanning system Leica TCS SP5 with inverted fluorescent microscope. All chemicals, except for RF, were purchased from Sigma-Aldrich. Data were analyzed by ANOVA.

RESULTS. Chromatin status and IFRF of 324 oocytes (in 3 replicates, 30-35 oocytes/group) were evaluated. Before culture level of IFRF in BCB^- oocytes was significantly higher than in BCB^+ oocytes (41±2.1 a.u. vs. 23±2.01 a.u., $P<0.05$). IFRF decreased both in BCB^+ and BCB^- oocytes after 36 hours of culture. The maximum level of IFRF in BCB^+ oocytes was observed after 15 hours and in BCB^- oocytes after 24 hours of culture (51±4.9 a.u. and 43±7.1 a.u.) at anaphase stage. There are no differences in the level of IFRF in BCB^+ (32±6,6 a.u.; 24 h of culture) and in BCB^- (31±5,4 a.u.; 30 h of culture) oocytes at metaphase stage.

CONCLUSION. The obtained results indicate differences in the kinetic of actin cytoskeleton functioning in oocytes that have finished the growth phase in vivo or in vitro during IVC.







Representative images of nuclear staining of oocytes: a-diakinesis; b-telophase; c – metaphase II (DAPI). Visualization of actin by RF: d - oocyte with low level of IFRF; e – oocyte with high level of IFRF

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