EFFECT OF HIGHLY DISPERSED SILICA NANOPARTICLES ON THE FUNCTIONAL ACTIVITY OF ACTIN CYTOSKELETON IN NATIVE AND DEVITRIFIED BOVINE OOCYTES DURING IVM

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INTRODUCTION. Actin takes over various essential function during oocyte meiosis (UrajiJ, et al., J Cell Sci ,131 22: 1-6, 2018). Nanoparticles are widely used in various fields including reproduction. The mechanisms of the influence of highly dispersed silica nanoparticles (HDSns) on the functioning of intracellular organelles are still not clear.

THE AIM of the present study was to identified the effects of HDSns (Chuiko Institute of Surface Chemistry, Ukraine) on the functional activity of the actin cytoskeleton [the intensity of fluorescence of rhodamine-phalloidin (IFRF) conjugated with actin filaments] in dynamics of meiosis of native (unfrozen) and devitrified (DV) oocytes.

MATERIALS & METHODS. IFRF was evaluated in: native oocytes; native oocytes were cultured with 0.001% of HDSns; DV oocytes; DV oocytes pre-treated with 0.001% of HDSns before vitrification (20 min) and were cultured with 0.001% of HDSns. Vitrification was performed by equilibration of cumulus oocyte complexes (COCs) before IVM in: CPA1:0.7 M dimethylsulphoxide (Me2SO) +0.9 M ethylene glycol (EG), 30 sec; CPA2:1.4 M Me2SO + 1.8 M EG, 30 sec; CPA3:2.8 M Me2SO + 3.6 M EG + 0.65 M trehalose, 20 sec and loading into straws. After thawing COCs washed in 0.25 M,0.19 M and 0.125 M trehalose in TCM-199 and finally in TCM-199. COCs were cultured 24 h in TCM 199 + 10% (v/v) FCS + 50 ng/ml PRL with 10^6granulosa cells /ml. For assessment of IFRF fixed oocytes were incubated sequentially in rhodamine-phalloidin(RF, R415 Invitrogen, Moscow, Russia), 1 IU/ml, for 30 min to label actin (Fig.1). 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. All chemicals were purchased from Sigma-Aldrich (Moscow, Russia). Data were analyzed by one-way ANOVA using Sigma Stat (Ver. 2).

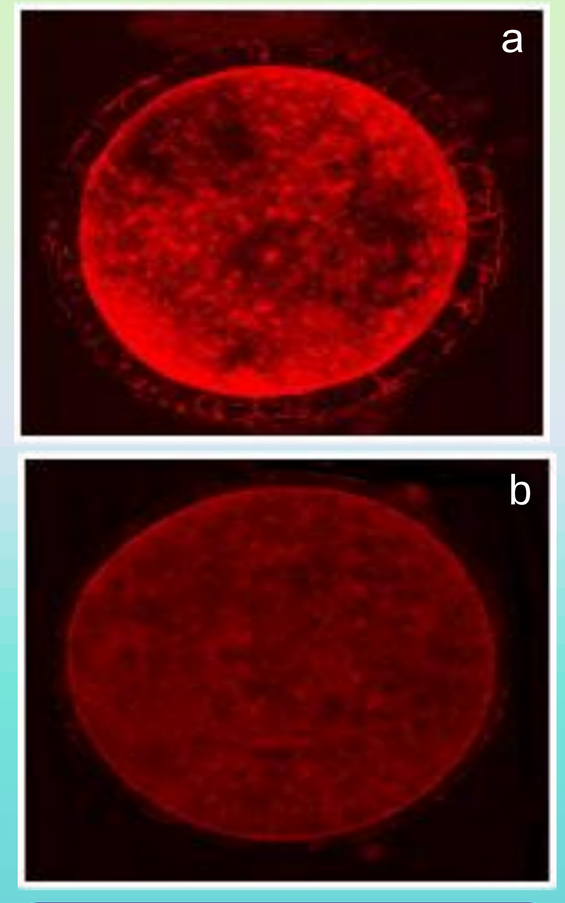
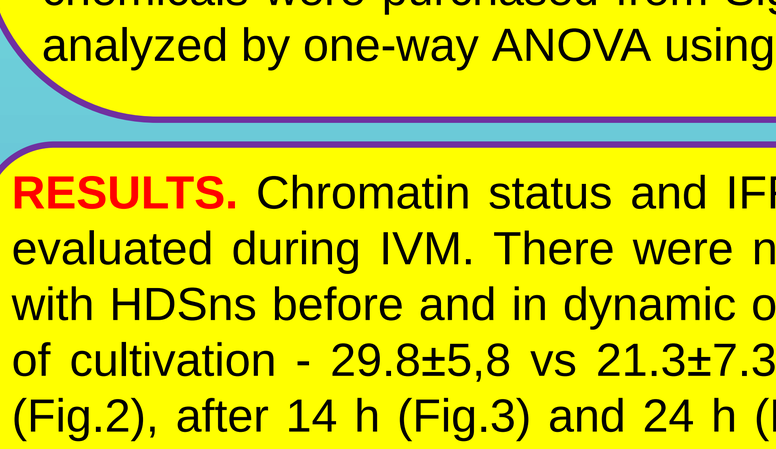


Fig.1 Visualization of actin by rhodamine-phalloidin:
a- oocyte with high IFRF;
b – oocyte with low IFRF

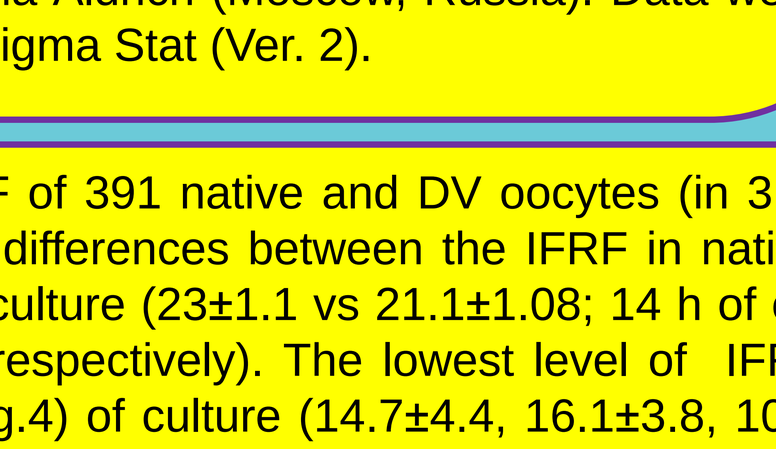
RESULTS. Chromatin status and IFRF of 391 native and DV oocytes (in 3 replicates, 30-34 oocytes/group) were evaluated during IVM. There were no differences between the IFRF in native oocytes and native oocytes treated with HDSns before and in dynamic of culture (23±1.1 vs 21.1±1.08; 14 h of cultivation - 55.8±5.6 vs 49.2±6,7; 24 h of cultivation - 29.8±5,8 vs 21.3±7.3, respectively). The lowest level of IFRF were tested in DV oocytes before (Fig.2), after 14 h (Fig.3) and 24 h (Fig.4) of culture (14.7±4.4, 16.1±3.8, 10.5±6.1, respectively). Treatment of DV oocyte with HDSns increased the IFRF after 14 h and 24 h of cultivation (16.1±3.8 vs 37.8±5.9 and 10.5±6.1 vs 23.5±4.9, respectively, $P<0.01$, $P<0.05$).

Fig. 2. IFRF in native and devitrified bovine oocytes before culture (n oocytes =139)



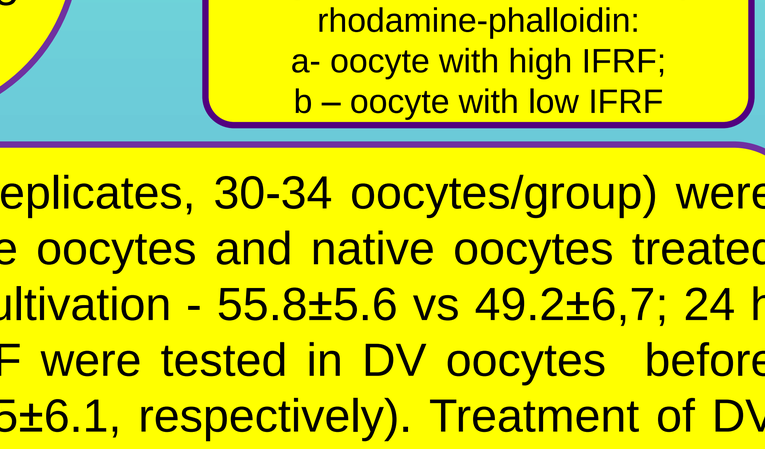
a:b;b:d$P<0.05$; b:c$P<0.01$

Fig. 3. IFRF in native and devitrified bovine oocytes after 14 h of culture (n oocytes =127)



a:d; b:d; c:d$P<0.05$; a:b;b:c$P<0.01$

Fig. 4. IFRF in native and devitrified bovine oocytes after 24 h of culture (n oocytes =125)



a:b; a:c a:d; b:d; c:d$P<0.05$; b:c$P<0.01$

CONCLUSION. The data of study showed that the treatment of COCs with 0.001% of HDSn influences on actin cytoskeleton integrity of bovine oocytes during vitrification. The mechanisms of the realization of this effect are under the further investigation.

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