Activity of maturation promoting factor and microtubule-activated protein kinase in frozen thawed human oocytes

V. Satta, L. Bogliolo[1], A. Borini[2], G. Coticchio[2], S. Succu, S. Ledda[1], F. Berlinguer, G.G. Leoni[3], S. Naitana

Department of Animal Biology, University of Sassari, Italy
[1]Department of Pathology and Veterinary Clinic, University of Sassari, Italy
[2]Tecnobios Procreazione, Centre for Reproductive Health, Bologna, Italy
[3]Department of Physiological Biochemical Cellular Sciences, University of Sassari, Italy

Following cryopreservation, the developmental ability of the human oocyte may be affected by several forms of cell damage, including perturbances in the level of regulatory proteins. Maturation promoting factor (MPF) and microtubule-activated protein kinase (MAPK) are two key regulators of the meiotic and mitotic cell cycles that ensure meiotic arrest, normal spindle configuration and chromosome condensation at the metaphase II (MII) stage. The aim of this study was to examine the biochemical activity of MPF and MAPK in frozen-thawed human mature oocytes.

Surplus human oocytes were donated by consenting IVF patients. Following cumulus cells removal, oocytes showing normal morphology and an extruded first polar body were placed in 2 1 of ice-cold collection buffer (1 mg/ml PVA, 5 mM EDTA, 10 mM Na_3VO_4, 10 mM NaF) and stored at −80 °C (control), or cryopreserved by slow cooling. For cryopreservation oocytes were exposed to 1.5 M propanediol and 0.2 mol/L sucrose before freezing. After thawing cryoprotectant dilution was performed in 0.3 M sucrose solution containing decreasing concentrations of propanediol. After 1 or 2 hours of in vitro culture, oocytes were put into 2 µl of ice cold collection buffer and stored at -80°C storage abd brought to a final volume of 9 µl with a solution containing 45 mM β-glycerophosphate, 12 mM *p*-nitrophenylphosphate, 20 mM MOPS-KOH, 12 mM $MgCl_2$, 12 mM EGTA, 0.1 mM EDTA, 1 mM DTT, 2.3 mM $NaVO_4$, 2 mM NaF, 0.8 mM PMSF, 15 µg/ml leupeptin, 30 µg/ml aprotinin, 0.1% (w/v) PVA, 1 mg/ml histone H1 (type III-S from calf thymus), myelin basic protein (1mg/ml, MBP), 2.2 M protein kinase inhibitor peptide, and 2.5 MBq/ml γ-[^{32}P]-ATP. The reaction was started after the addition of γ-[^{32}P]-ATP and carried out for 30 min at 37°C.

Phosphorylation of artificial substrates histone H1 and MBP were considered as signs of MPF and MAPK activity, respectively. Proteins were separated on 1D SDS-PAGE electrophoresis and radioactive bands were analyzed by gel autoradiography. The mean pixel intensity of a preselected set area was measured using Kodak Image Analysis Software 1D 3.6. The mean intensity of the bands of fresh controls were assumed to correspond to 100 arbitrary units and the mean band intensities of the other groups were quantified comparatively to this value. Analysis of variance (ANOVA) was used to assess the significance of differences in MPF and MAPK activity among groups. $P<0.05$ was considered significant.

In frozen-thawed oocytes that where cultured for 1 hour after thawing, the relative intensity (RI) of the MPF bands was not statistically different (98.2) compared to the fresh control. However, MPF band intensity was significantly reduced (RI=73.3, $P<0.05$) in samples cultured for 2 hours after freezing-thawing. Conversely, comparable intensities of the MAPK bands were observed in control and cryopreserved oocytes cultured for 1 or 2 hours (RI=100, 96.8, and 98.7, respectively).

In human oocytes slow-freezing appears to partly influence the activity of cell cycle regulatory proteins. After thawing, MAPK activity is unaffected over a period of two hours. During the same interval, MPF activity is initially maintained unaltered but undergoes a significant decrease thereafter. This may have significant implications for the use of frozen-thawed oocytes. In particular, considering the observed delayed reduction in MPF activity, it might be appropriate to limit to 1 hour the period of post-thaw culture before ICSI, thereby preventing possible losses in spindle and chromosome configuration, or premature exit from the MII arrest.