Potassium Assay in Blastomere of Two-Cell Mouse Embryo after Equilibration and Washing from Cryoprotector

D. V. Gol'dshtein, E. I. Smol'yaninova, and A. G. Pogorelov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 7, pp. 48-49, July, 2004
Original article submitted April 21, 2004

The need for effective methods of cryopreservation of early mammalian embryos necessitates the study of the mechanisms of blastomere adaptation to the equilibration procedure and subsequent washing from the cryoprotector. The osmotic effects during these procedures can cause electrolyte imbalance in embryonic cells. Intracellular potassium concentrations at the stage of two-blastomere mouse embryo were studied by electron probe microanalysis.

Key Words: *cryopreservation; ethylene glycol; two-blastomere embryo; cytoplasmatic potassium; electron probe microanalysis*

Freezing in liquid nitrogen is an effective method for cryopreservation of early mammalian embryos [5]. Before freezing the cytoplasm of embryonic cell is equilibrated with extracellular environment containing a mixture of cryoprotectors. Pre-equilibration reduces the probability of functional injuries induced by subsequent plunging of the embryo into liquid nitrogen. Viability of defrosted embryos depends on equilibration procedure [1,2]. Possible causes of this dependence are osmotic phenomena and disorders in potassium homeostasis. Creation of effective methods for cryopreservation puts forward the problem of intracellular potassium monitoring during equilibration and subsequent washing from cryoprotectors. We developed a method for electron probe microanalysis of potassium concentration in the blastomere of two-cell embryo.

MATERIALS AND METHODS

Experiments were carried out on two-blastomere embryos of SHK mice. All manipulations with the embryo were carried out at 20°C. The treatment of the embryo started with 10-min equilibration in 10% aqueous solution of ethylene glycol (EG). Then the embryos were plunged for 1.5 min into freezing medium (30% EG in 1 M sucrose solution). Preliminary preparation to freezing was thus over. The next step was removal of EG in 0.5 M sucrose solution (10 min) with subsequent washing in Dulbecco's medium (20 min). All solutions used in the study were prepared on Dulbecco's medium.

Intracellular potassium concentration was measured by electron probe microanalysis in a section of a two-cell embryo prepared as described previously [3,4]. The sample was cryofixed in liquid propane followed by vacuum lyophilization at 200°K. Dehydrated embryo was embedded into resin, which was polymerized at 330°K. The sections (2 μ) of the sample embedded in resin were analyzed under a JSM-U3 scanning electron microscope (JEOL). Electron probe microanalysis of potassium concentration in the blastomere cytoplasm was carried out at accelerating voltage of 25 kV, probe electron current of 5 nA, the duration of analysis was 20 sec.

RESULTS

Electron probe microanalysis of potassium concentration in the cytoplasm of two-cell embryo blastomere

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino. ***Address for correspondence:*** pogorelov_a@mail.ru, pogorelov@iteb.ru. Pogorelov A. G.

0007-4888/04/1381-0040

TABLE 1. Potassium Concentration in Blastomere Cytoplasm of Two-Cell Mouse Embryo in Control and after Ethylene Glycol Equilibration/Washout (Experiment; *M±SEM*)

Group	Potassium, mM	*n*
Control	130±6	7
Experiment	47±3	9

Note. *n*: number of embryos in the group.

showed that the cytoplasmic potassium concentration in mature two-cell mouse embryos (Table 1) is close to that observed in specialized cells [3,4]. As a result of cryopreservation, the concentration of intracellular potassium decreased from 130±6 mM to 47±3 mM. Hence, the protocol of the cryoprotector (ethylene glycol) equilibration/washout leads to imbalance of potassium homeostasis in cells of two-blastomere embryos, which can cause subsequent developmental disorders.

REFERENCES

1. A. S. Krivokharchenko, L. I. Vil'yanovich, and G. A. Serobyan, *Probl. Reproduktsii*, **4**, 13-17 (1995).
2. E. I. Smol'yaninova, O. B. Khromenkova, and G. V. Zhernoklev, *Probl. Kriobiol.*, **2**, 49-55 (2001).
3. A. Pogorelov, B. Allachverdov, I. Burovina, *et al.*, *J. Microsc.*, **162**, Pt. 2, 255-269 (1991).
4. A. Pogorelov, V. Pogorelova, N. Repin, and I. Mizin, *Scanning Microsc. Suppl.*, **8**, 101-108 (1994).
5. L. Wilson and P. Quinn, *Hum. Reprod.*, **4**, 86-90 (1989).