Estimation of Isotonic Point of Incubation Medium For Two-Cell Mouse Embryo

M. A. Pogorelova[1], V. A. Golichenkov[2], V. N. Pogorelova[1], E. V. Kornienko[1,3], A. I. Panait[1], and A. G. Pogorelov[1,3]

Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 3, pp. 155-158, September, 2011
Original article submitted April 28, 2011

Osmolarity of Dulbecco's medium at which the volume of two-cell mouse embryo remained similar to that of intact embryo was determined. The method is based on comparison of kinetic curves describing the volume of embryonic cell in solutions of different osmolarity. The blastomere volume was measured by quantitative laser microtomography after fixed osmotic stress intervals. It was found that Dulbecco's saline with 125 mM NaCl solution is an isotonic solution for two-cell mouse embryo. This concentration corresponds to 290 mOsm, which is lower than osmolarity (~310 mOsm) of media routinely used for culturing of differentiated cells or biological fluids, *e.g.* blood plasma.

Key Words: *two-cell mouse embryo; osmotic stress; blastomere volume; laser scanning microscopy; 3D reconstruction*

Early embryogenesis in mammals is extremely sensitive to anisotonic conditions *in vitro* [5,9,10]. Hypotonic shock (180 mOsm) leads to arrest of the first cell cycle, while hyperosmotic stress blocks zygote development at approximately 410 mOsm [8]. It is empirically demonstrated that the medium with osmolarity of 260 mOsm by the survival criterion is optimal for culturing of one-cell embryo [11], which is considerably below the physiological level for many biological fluids, *e.g.* blood plasma (~310 mOsm).

Analytic measurements of physicochemical parameters of fluid from the oviduct lumen are limited by relatively low volume of this compartment. The data of electron probe microanalysis of the concentration of the main biologically important elements in the luminal fluid suggest that osmotic properties vary in different compartments of the reproductive tract [6]. According to our measurements, osmolarity of mouse oviduct fluid is 360 mOsm. This high value is probably a result of certain approximations during calculation of this parameter. This, estimation of the isotonic point of the solution typical of each stage of embryo development is an urgent problem.

For one-cell mouse embryo, a method was developed based on comparison of kinetic curves describing changes in zygote volume in response to osmotic stress of different direction and magnitude [8]. The authors assumed that in isotonic medium the embryo does not change its volume, *i.e.* it does not swell or shrink in comparison with its initial volume. The authors proceeded from the following assumptions: spherical shape of the cell, spatial isotropy, and known mathematic relation between cross-section diameter measured under a microscope and the volume of one-cell embryo.

These assumptions are incorrect for two-cell embryo; it has no spherical asymmetry.

Our aim was to adapt the concept proposed for zygote to a multicellular system of early embryo. The cell volume was measured by quantitative laser microtomography (LMT) allowing volume measurements for objects (blastomere) with complex spatial structure [2-4,14].

[1]Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino; [2]Biological Faculty, M. V. Lomonosov Moscow State University; [3]Pushchino State University, Russia. ***Address for correspondence:*** agpogorelov@rambler.ru. A. G. Pogorelov

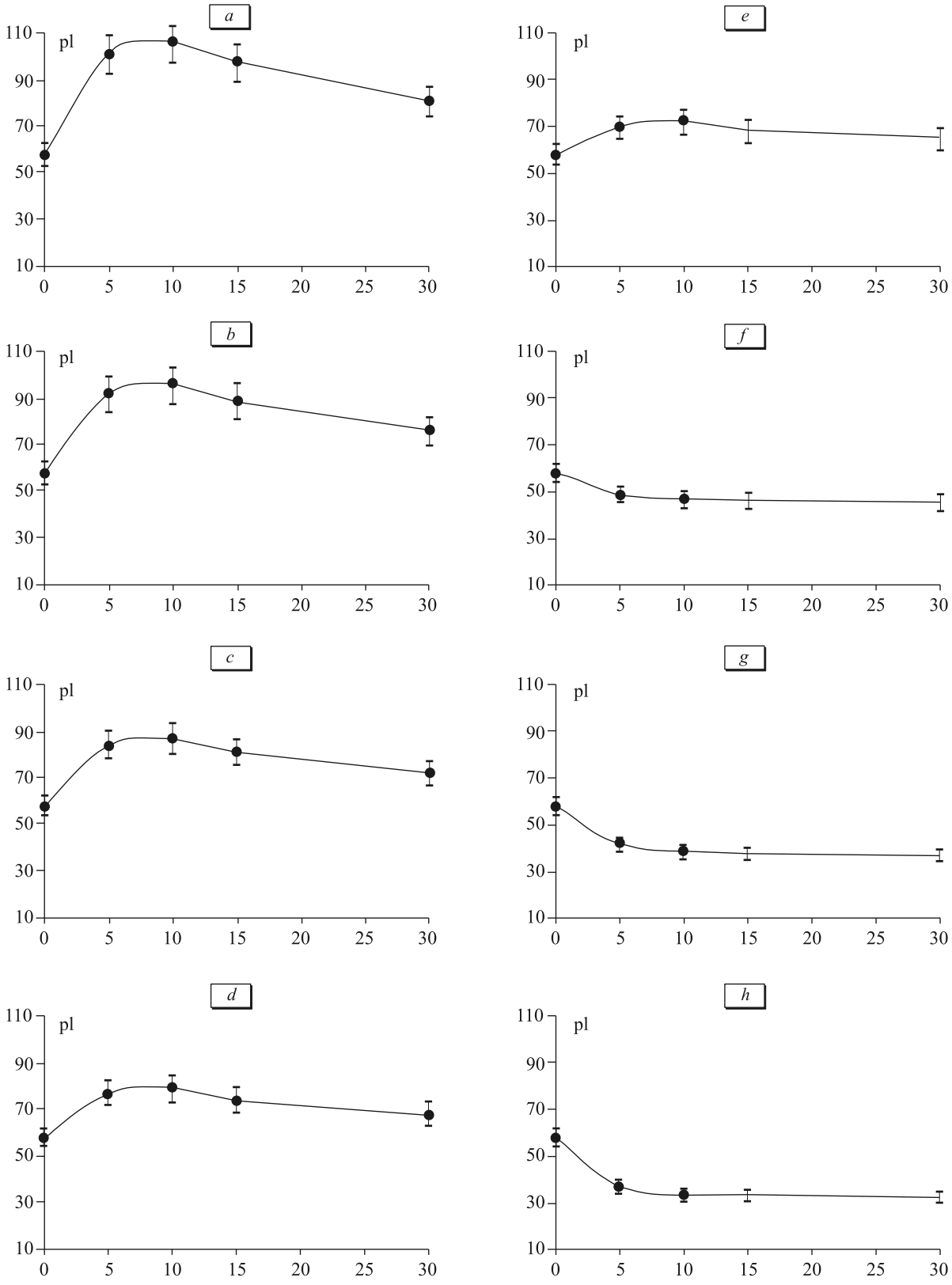

Fig. 1. Changes in two-cell mouse embryo blastomere volume during 30-min osmotic stress of different magnitude and direction (osmolarity of the solution: *a*) 150 mOsm, *b*) 170 mOsm, *c*) 190 mOsm, *d*) 210 mOsm, *e*) 230 mOsm, *f*) 370 mOsm, *g*) 430 mOsm, *h*) 470 mOsm). Abscissa: time of stress exposure (min). Initial point: volume of the embryonic cell immediately after extraction from the oviduct. The data are presented as the mean and standard deviation of the mean for the group ($n \geq 20$ embryos).

MATERIALS AND METHODS

Experiments were carried out on SHK mice obtained from vivarium of Institute of Theoretical and Practical Biophysics, Russian Academy of Sciences (Pushchino). The animals were kept under standard conditions (20±2°C, pelleted food PK-121-2, water *ad libitum*). Water, fodder, and bedding were changed daily without sterilization. Two-cell mouse embryos were isolated as described elsewhere [1]. The cells were incubated in Dulbecco's medium. Osmotic shock was modeled by varying NaCl concentration in the incubation medium. Embryos analyzed immediately after their extraction from the oviduct served as the control.

The principles of specimen preparation based on snap cryofixation of biological tissue were described previously [12,13]. The initial stage is cryofixation in liquid propane (-188°C). The frozen embryos were lyophilized under vacuum (10^{-3} Pa) at low temperature (-100°C) using MBA 5 freeze drier (Balzers), and embedded into Epon 812 [12].

The volume of individual blastomeres was measured by quantitative LMT [2-4,14]. The specimens were then examined under a Leica TCS scanning microscope (Leica). A vertical stack of optical sections in transmitted light with a 1-μ step was obtained. Taking into account low contrast of the obtained digital image, each section was additionally processed by a unified algorithm using Adobe Photoshop 6.0 or GIMP 2.2.17 software. The blastomere contours in each optical section were outlined and then spatial structure was reconstructed by a series of successive contours [7,14].

RESULTS

Difficulties in measurements of the blastomere volume in the multicellular system is the main obstacle in studies of the osmotic response of the early embryo, therefore the experiments were performed on mouse zygote taken as a sphere for simplification [8]. Spherical approximation allows calculation of the volume of an individual cell by measuring its radius and using the formula relating sphere volume and its radius. This approach can not be applied even for relatively simple two-cell embryo system consisting of non-spherical blastomeres.

We developed a LMT-based technology of computer modeling of a spatial image of an individual blastomere [3]. In respect to embryonic cell, the 3DR approach allows not only visualization of a complex object, but also evaluation of its volume characteristics.

We incubated the isolated embryo at 150-470 mOsm. The volume of the embryonic cell immediately after its extraction from the oviduct into the Dulbecco's solution was taken as the initial (control) value. In this case, the time elapsed after embryo isolation to its cryofixation did not exceed 2 min. According to quantitative LMT data, we constructed kinetic curves for measuring the volume of blastomere of a two-cell mouse embryo under conditions of osmotic stress of different magnitude and direction (Fig. 1).

The blastomere volume initially increased in media with osmolarities of 150-230 mOsm. Ten minutes after the start of exposure, this parameter attained a maximum and started to recover. In hyperosmotic media (370-470 mOsm), the embryonic cell shrank and after 10 min its volume remained constant (Fig. 1).

It is assumed that the volume of the embryo during the very first minutes of incubation in a medium with osmolarity close of physiological value little differs from the volume of intact embryo. Semiquantita-

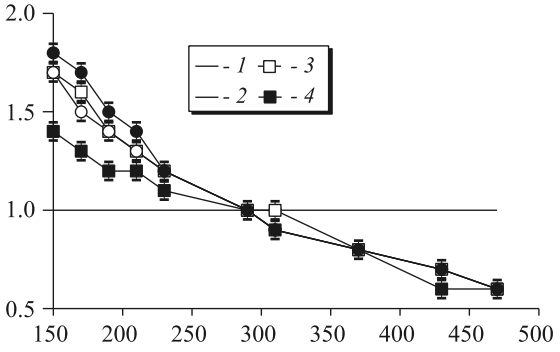

Fig. 2. The dependence of relative volume (ordinate) of two-cell mouse embryo blastomere on medium osmolarity (abscissa) at different terms of incubation in Dulbecco's medium. Horizontal line corresponds to relative volume of embryonic cell under isotonic conditions. *1*) 5 min, *2*) 10 min, *3*) 15 min, *4*) 30 min.

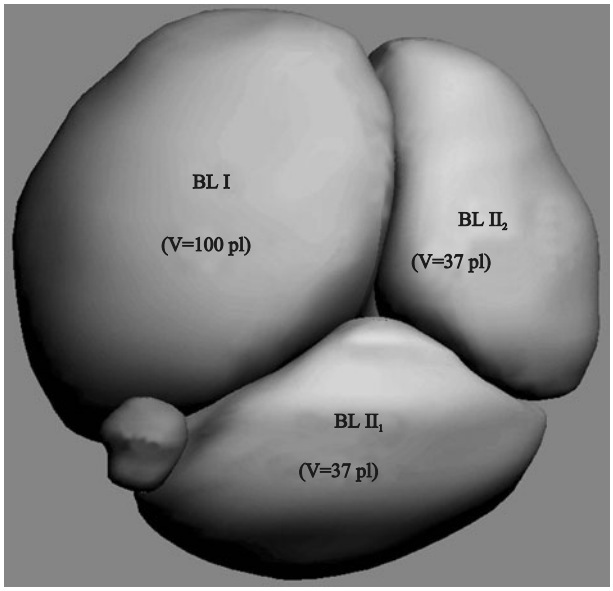

Fig. 3. Early mouse embryo at the stage of 3 blastomeres: image obtained by semiquantitative LMT. BL: blastomere, PB: polar body, V: blastomere volume.

tive evaluation showed that the volume of a two-cell mouse embryo is about 300 mOsm [8]. Under hypotonic conditions, the cell volume increases and under hypertonic decreases in comparison with the initial state. Only in the true isotonic saline, the cell volume remains unchanged, *i.e.* the ratio of actual blastomere volume at any incubation term to its initial volume is 1.0. To analyze the dependence of the relative volume on osmolarity, we constructed kinetic curves of volume changes in the corresponding coordinates (Fig. 2).

The point of intersection of the curve with the straight line corresponds to isotonic medium where the embryo volume does not change with time (Fig. 2). This is true for a solution with osmolarity of 290 mOsm. Semiquantitative method (evaluation of blastomere reaction to osmotic stress by measuring its linear size) yields similar value [8] . Thus, the data of semiquantitative LMT are comparable with the results of visual control of osmotic behavior of a two-cell embryo.

It should be noted that changes in geometrical parameters of the cell at later stages of embryo development, i.e. when its spatial structure becomes more complex, are a problem for routine light microscopy, but not for 3DR, when it used for direct measurement of the volume of blastomere of any shape. That is why the method of semiquantitative LMT can also be used at later stages of early embryogenesis (Fig. 3).

The accuracy of measurement of cell volume measurement by using 3DR is determined by the number of optical sections of the object, *i.e.* spatial resolution of laser scanning microscope in vertical direction. Form this viewpoint, the advantage of two-cell embryo is its relatively large size. Our experience in using Zeiss and Leica devices suggests that the method can be applied even to 16-cell embryo, where the size of embryonic cell is comparable to that of somatic cell. Thus, the technology of quantitative LMT can be applied to studies of differentiated cell cultures.

REFERENCES

1. O. P. Berezovskaya, L. M. Mezhevikina, and B. N. Veprintsev, *Ontogenez*, **17**, 553-555 (1986).
2. A. G. Pogorelov and V. N. Pogorelova, *Biofizika*, **54**, 482-487 (2009).
3. M. A. Pogorelova, D. V. Gol'dstein, A. G. Pogorelov, and V.A. Galichenkov, *Kletochn. Tekhnol. Biol. Med*., No. 3, 169-172 (2009).
4. M. A. Pogorelova, D. V. Gol'dstein, A. G. Pogorelov, and V.A. Golichenkov, *Dokl. Akad. Nauk*, **418**, No. 5, 712-714 (2008).
5. J. M. Baltz and A.P. Tartia, *Human Reprod. Update*, **16**, No. 2, 166-176 (2010).
6. R. M. Borland, S. Hazra, J. D. Biggers, and C. P. Lechene, *Biol. Reprod.,* **16**, No. 2, 147-157 (1977).
7. A. Y. Budantsev and Y. Y. Yakovlev, *Eur. Microscopy Analysis.* No. 9, 11-14 (2000).
8. J. L. Collins and J. M. Baltz, *Biol. Reprod.,* **60**, No. 5, 1188-1193 (1999).
9. A. Davidson, M. Vermesh, R. A. Lobo, and R. J. Paulson, *J. in Vitro Fert. Embryo Transfer.,* **5**, No. 3, 149-152 (1988).
10. A. Hay-Schmidt, *J. Assist. Reprod. Genet.,* **10**, No. 1, 95-98 (1993).
11. J. A. Lawitts and J. D. Biggers, *Guide to Techniques in Mouse Development*, Eds. P. M. Wassarman and M. L. DePamphilis, San Diego, CA (1993), pp. 153-164.
12. A. G. Pogorelov, B. L. Allachverdov, I. V. Burovina, *et al., J. Microscopy*, **12**, 24-38 (1991).
13. A. G. Pogorelov, I. I. Katkov, E. I. Smolyaninova, and D. V. Goldshtein, *Cryo Letters,* **27**, No. 6, 87-98 (2006).
14. A. G. Pogorelov and V. N. Pogorelova, *J. Microsc.,* **232**, No. 1, 36-43 (2008).