UDC 591.392

DEVELOPMENT OF EARLY *IN VIVO* MOUSE EMBRYOS AT DIFFERENT VOLUME OF CULTURAL DROP

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Method of maturation and insemination of oocytes *in vitro* is an alternative way of obtaining embryos for transplantation. This technique is already used to increase in the population of the part of animals with desirable genotype and to accelerate the rate of selection [Kasinathan P. et al., 2015]. But the quality of *in vitro* embryos remains worse than of embryos derived with MOET-technology. Therefore optimization of technological aspects of the culture procedure is actual task. In order to determine conditions that promote the full development of early embryos *in vitro*, dependence of their development on the volume of the culture drop had been studied.

In experiments the *in vivo* 2-cell embryos from 2–2,5 month old white laboratory mice were used. To obtain embryos the animals stimulated with PMSG (10 IO, "Folligon", "Intervet", Holland) and hCG (10 IO, "Pregnil"), mated with males and killed by vertebrae displacement at 40–42 h after injection of luteinizing hormone. The study was conducted with 5 repetitions with use in each 3–4 animals. To keep the "pair-analogues" principle at each repetition the embryos from all animals were mixed and then were divided into groups. Embryos were cultured at the two-step scheme. In the first stage (IVC1) fresh 2-cell *in vivo* embryos were transferred to M16 medium with salts according to M. Mank (1990), which added HEPES (2 mg/ml, "Sigma"), sodium pyruvate (0,1 mg/ml, P4562, "Sigma"), bovine serum albumin (4 mg/ml, A3311, "Sigma"), mixtures of minimal (MEM, M7145, "Sigma") and basal (BME, B6766, "Sigma") amino acids listed by H. Eagle (1% v/v of each), glutamine (0,1 mg/ml, "Reachim"), a mixture of Eagle's vitamins (1% v/v, M6895, "Sigma"). The size of the culture drop was 0,1 or 0,5 ml, the number of embryos in drop — 10,2±0,22 ones. After 48 h the cultured embryos were transferred in the drop with volume similar to first stage in the medium of analogical composition but supplemented with glucose (1 mg/ml, G7021, "Sigma"), and were cultured for next 48 h (stage IVC2). Time from injection of hCG to embryos extraction averaged to 40,8±0,6 h, to transfer embryos into the medium IVC2 — 91,4±1,0 h.

Reducing of volume of drop and increasing of density of embryos do not affect the first division — the amount of 4-cell embryos in both cases was the same. The difference between technological options appeared later. So, the proportion of embryos that after 48 h formed a compacted morula in the group with higher density (0,1 ml) was non-credibly lower. Number of blastocysts observed after 72 h culture was also significantly lower in the group with a drop volume 0,1 ml. After 96 h culture the difference in the number of observed blastocysts between the options remained but became non-credible (Tab.).

Table

Development of 2-cell mouse embryos at different volume of culture drop

Volume of IVC1 drop/ IVC2 drop, ml	N/n	Proportion of embryos on stage after hours of culture, %			
		4-cell after 24 h	MC after 48 h	Bl after 72 h	Bl after 96 h
0,1/0,1	5/51	82,0±8,2^{a}	48,5±12,3^{a}	19,5±6,9^{a}	36,7±10,9^{a}
0,5/0,5	5/51	82,0±8,9^{a}	58,5±9,2^{a}	44,7±8,1^{b}	58,5±6,9^{a}

Notes: 1) N — the number of repetitions, n — the number of cultured embryos. 2) Abbreviation used: 4-cell — embryo with 4 blastomeres, MC — morula compacted, Bl — blastocyst; parameters in same column with different subscripts differ with variance not more 0,05.

The reducing of drop volume with a corresponding increase of embryo density worsened quality of germs. So, proportion of 4-cell embryos developed after 24 h to the compacted morulae stage in group with 0,1 ml drop volume was 58,0±13,5%, while with the increasing of the volume enhanced to 71,3±8,7% ($p > 0,05$). Proportion of compacted morula that after 24 h developed to the blastocyst stage was 35,8±14,6 and 78,1±9,3% accordingly ($p < 0,05$), while quantity of morula that formed blastocyst after 48 h IVC2 — 82,5±17,3 and 106,5±17,4% accordingly ($p > 0,05$).

Thus, reducing the drop volume with increasing embryo density leads to a retardation of their development and decreases the final effectiveness of cultivation.

The results of the experiment will be used to improve methods of obtaining the *in vitro* embryos of farm animals.