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EFFECT OF VITAMINS ON DEVELOPMENT OF EARLY *IN VIVO* MOUSE EMBRYOS CULTURED *IN VITRO*

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As a model for studying embryogenesis in mammals often use the mouse embryos. The obtained data not only deepen the knowledge of biological mechanisms, but also shows the direction of further improvement of reproductive *in vitro* technology. The first stages of differentiation of germ cells are the processes of compaction and cavitation. The success of their overcoming makes possible further morphogenesis in embryos. Therefore, the conditions that contribute to the initiation and passage of these processes are interesting both in theoretical and practical terms. The aim of this study was to examine the effect of vitamins addition to culture medium on the development of early mice embryos. Vitamins and their concentration in the experimental mediums corresponded to those in the Eagle's culture medium (Eagle H., 1955).

In experiments the 2-cell *in vivo* 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 3 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 accordingly to the two-step scheme. For this the fresh-derived 2-cell embryos were transferred to 0,1 ml experimental solution and cultured for 48 h (stage IVC1). Then the embryos were transferred in 0,1 ml medium of the second stage (IVC2) and kept for next 48 hours. The basis of the culture medium was SOF solution with inorganic salts listed H.R. Tervit (1972) and supplemented with HEPES (2 mg/ml, "Sigma"), sodium pyruvate (0,1 mg/ml, P4562, "Sigma"), sodium lactate (3,12 mg/ml, L4263, "Sigma"), bovine serum albumin (1 mg/ml, A3311, "Sigma"), mixtures of minimal (MEM, M7145, "Sigma") and basic (BME, B6766, "Sigma") amino acids listed by H. Eagle (1% v/v of each), glutamine (0,1 mg/ml, "Reachim"). The mixture of Eagle's vitamins (M6895, "Sigma") was added to the experimental medium in the amount 1% v/v, D-glucose (G7021, "Sigma") — 1,0 mg/ml. Time from injection of hCG to embryos extraction averaged to 42,0±0,7 h, to transfer embryos into the medium IVC2 — 88,0±0,4 h.

Supplement of medium of first stage of cultivation by vitamins improved development of the embryo that appeared in increase of proportion of embryos that after 24 h had 4 or more blastomeres and which were on compacted morula stage after 48 h and developed to the blastocyst stage after 96 h (Table.). However, the lack of vitamins in the first stage had not prevented the several embryos develop to the compacted morula, and therefore is not a limiting factor for the compaction of blastomeres. It should be noted that in our previous studies, not combined with these experiments, formation of blastocoel in embryos were observed in the absence of vitamins for the second stage, but with the obligatory addition of glucose. The latter indicates that vitamins listed by H. Eagle is not a determining factor in beginning the process of cavitation.

Table

Effect of vitamins on development of 2-cell *in vivo* embryos in mice

№	Added substances		N/n	Proportion of embryos on stage after hours of culture, %			
	in IVC1	in IVC2		4-cell after 24 h	MC after 48 h	Bl after 72 h	Bl after 96 h
1	-	Vit	3/30	53,3±35,6	26,7±21,6	0,0	0,0
2	-	Vit, Gl	3/31	47,0±32,2	37,3±29,3	10,0±12,3	16,1±10,7
3	Vit	Vit	3/29	64,8±16,4	57,8±26,0	3,3±4,1	10,0±12,3
4	Vit	Vit, Gl	3/30	63,3±10,8	60,0±21,2	6,7±4,1	20,0±7,1
	-	Vit/Vit, Gl	6/61	50,2±19,3	32,0±14,8	5,0±5,5	8,0±5,8
	Vit	Vit/Vit, Gl	6/59	64,1±7,9	58,9±13,4	5,0±2,5	15,0±6,2

Notes: 1) N — the number of repetitions, n — the number of cultured embryos. 2) Abbreviation used: Vit — vitamins, Gl — glucose, 4-cell — embryo with 4 blastomeres, MC — morula compacted, Bl — blastocyst.

So, addition of a mixture of Eagle's vitamins in medium improves the development *in vitro* of 2-cell *in vivo* embryos of mice, but their absence not limits the beginning of a process of compaction of blastomeres in embryos.