UDC 591.392

ROLE OF GLUCOSE IN INITIATION OF COMPACTION AND CAVITATION IN EARLY MOUSE EMBRYOS IN CULTURE *IN VITRO*

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The role of glucose in the development of in vitro embryos is ambiguous. So, the presence of carbohydrate in CZB medium inhibited the development of 1–4-cell mouse embryos in vitro, but was necessary after the 4-cell stage to be possible for embryos grow to the blastocysts [Chatot C.L. et al., 1989]. Addition 2,5–10 mM of this substance in the medium with defined composition improved in vitro development of pig zygotes [Mito T. et al., 2012]. At the same time, the addition of glucose inhibited the development of IVP-morula and blastocyst in cows and drifted the distribution of descendants towards males [Kimura K. et al., 2005]. Some results suggested that the increased amount of glucose in a medium is responsible for the 8-16-cell block of development of cow embryos [Takahashi H., First N.L., 1993]. So, to develop optimal conditions of in vitro culture the investigation of the action of glucose at all stages of embryogenesis is a key task. The aim of the study was to determine the role of glucose in initiation of process of compaction and cavitation in early mice embryos cultured in vitro.

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 41–43 h after injection of luteinizing hormone. 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 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"). D-glucose (G7021, "Sigma") was added to the solution in an amount of 5,5 mM (1,0 mg/ml). Time from injection of hCG to embryos extraction averaged to 42,5±1,2 h, to transfer embryos into the medium IVC2 — 87,5±1,8 h.

The data confirmed opinion that the presence of glucose in the solution is a prerequisite for initiation of cavitation process in the embryos. So, in the absence of carbohydrate in the medium IVC2 none of the embryos developed to the blastocyst (Table.). However, the addition of carbohydrate not proved necessary to initiate the process of compaction of blastomeres in embryos too. Also it was not observed of the negative influence of glucose on 2-cell *in vivo* embryos. On the contrary, the addition of carbohydrate in the first phase of cultivation improved the ability of embryos to the initiation of compaction and subsequent formation of blastocoel.

Table

Effect of glucose (1 mg/ml) on *in vitro* development of 2-cell *in vivo* embryos of mice

№	Addition of glucose		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	-	-	4/65	74,3±15,5[a]	44,8±14,3[a]	0,0[a]	0,0[a]
2	-	+	4/66	68,8±20,5[a]	54,8±17,1[a]	21,3±15,7[a,b]	26,1±13,9[a,b]
3	+	+	2/19	89,5±0,8[a]	74,5±20,4[a]	26,7±9,4[b]	31,7±2,3[b]

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.

Our results are consistent with those of other authors on the role of glucose in the initiation of cavitation [Brown J.J.G., Whittengham D.G., 1991; Pantaleon M. et al., 2008], but not with the conclusion that to form blastocoel embryos must necessarily be endure for some time in medium with this carbohydrate prior to the beginning of compaction of blastomeres [Chatot C.L. et al., 1994]. It should be noted that in parallel our studies the single cases of initiation of cavitation was observed in compacted morula when IVC1 and IVC2 mediums not contained glucose, but were complemented by Eagle's vitamins. Some authors explain these cases of obtaining of blastocysts in mediums without glucose by increased consumption of other energy substances, including pyruvate [Martin K.L., Leese H.J., 1995].

Consequently, it was confirmed the role of glucose in initiating the process of cavitation in the early embryos of mice.

Further research will be aimed at finding the optimal content of carbohydrates in the culture medium.