Lucrări ştiinţifice **Zootehnie şi Biotehnologii**, vol. **42 (1)** (**2009**), Timişoara

RESEARCHES REGARDING THE INFLUENCE OF RECOVERY MEDIA ON THE IN VITRO DEVELOPMENT CAPACITY OF THE PREIMPLANTATIONAL MOUSE EMBRYO

CERCETĂRI PRIVIND INFLUENŢA MEDIULUI DE RECOLTARE ASUPRA CAPACITĂŢII DE DEZVOLTARE IN VITRO A EMBRIONILOR PREIMPLANTAŢIONALI DE ŞOARECE

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Phosphate Bufered Saline with 0.4% BSA and M2 medium are one of the most common media used in embryo recovery. The aim of our paper was to investigate if the recovery media used for the recovery of the mouse embryo is influencing in vitro developmental capacity. As biological material we used 10 used were mouse females, age 2 months superovulated with 5UI PMSG (Pregnant Mare Serum Gonadotropine) and 5 UI hCG (human Corionic Gonadotropine). The embryos used were recovered, by oviduct flushing, at 24 hours from the identification of the vaginal plug. The majority of the embryos (78.3%) were in two cells stage. A total of 123, 2 cells embryos were cultivated in M16 medium. The evolution of the embryos was examined at 24, 48 and 72 hours interval. The proportion of hatched blastocyst was higher at the embryos recovered with M2 (53.7%) compared with the embryos recovered with PBS 0.4% BSA. The difference is statistically very significant ($p<0.001$). Embryos recovered in M2 media have a higher in vitro developmental capacity compared with the embryos recovered in PBS media supplemented with 0,4% BSA, possibly because of the sodium bicarbonate and lactate used in M2 media for pH regulation.

Keywords: embryos, recovery media, M2, PBS-0,4%BSA, hatching rate

Introduction

Phosphate Bufered Saline with 0.4% BSA and M2 medium are one of the most common media used in embryo recovery. Toru Takeo (2009) showed that PBS and M2 remained at a neutral pH for 144 h, whereas mWM exhibited pH elevation after 24 h. The alteration of the pH, may cause alterations of the development, because it plays an important role in the maintenance of normal cell

functions (L.J. Edwards 1998). Moreover, impairment of pH regulation can cause cell death (D. Lagadic-Gossmann 2004).

A study performed by Schini et. al. (1988) showed that the blockage of embryo development at mice embryo is partial or totally due to the presence of glucose of phosphate in culture media.

The aim of our paper was to investigate if the recovery media used for the recovery of the mouse embryo is influencing *in vitro* developmental capacity.

Materials and Methods

As biological material we used 10 used were mouse females, age 2 months superovulated with 5UI PMSG (Pregnant Mare Serum Gonadotropine) and 5 UI hCG (human Corionic Gonadotropine). The protocol for superovulating the females is presented in figure 1.

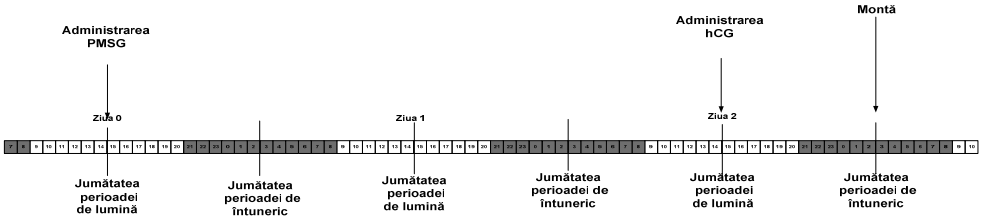

Figure 1. Superovulation inducing protocol

PMSG administration was perform at 15^{00} (half of the light period) in the 0 day of the superovulatoy treatment. hCG was administered in the second day of the protocol, at 48 hours from PMSG administration. After the last hormone administration the females were put with males for mating.

The next morning the vaginal plug was checked, and embryos were recovered at 24 hours after the vaginal plug.

The embryos used were recovered, by oviduct flushing, at 24 hours from the identification of the vaginal plug.

As recovery media we used 2 media:

1. a simple media PBS (Phosphate Bufer Saline) suplimented with 0,4% BSA (Bovine Seric Albumine);
2. a complex media M2, modified Krebs-Ringer solution with HEPES buffer substitute of the bicarbonate.

After recovery embryos were morphological evaluated and only the embryos in quality code 1 and 2 were *in vitro* cultivated in M16 media.

The evolution of the embryos was examined at 24, 48 and 72 hours interval.

The data were analyzed using chi-square test.

Results and Discussion

From the 10th females treated 7 were found with vaginal plug. The results obtained at the recovery of the embryos are presented in table 1.

Table 1

Results obtained at embryo recovery

Superovulated females N	Females with vaginal plug N	Embryos recovered		From which							
		N		ovocites		2 cells		4 cells		degenerated	
				n	%	n	%	n	%	n	%
10	7	157	21.9	11	7	123	78.3	13	8.3	10	6.4

From table 1 it can be noticed that from the 10 females taken into experiment 7 were found with vaginal plug. Total number of embryos recovered was 157 embryos which represents a mean of 21.9 embryos per female. From the embryos recovered 7% (n=11) were ovocytes, 78.3% (n=123) were 2 cells embryos, 8.3% (n=13) were 4 cells embryos and 6.4% (n=10) were degenerated embryos.

The majority (78.3%) of the embryos recovered at 24 incubation hours after the vaginal plug were in 2 cells stage.

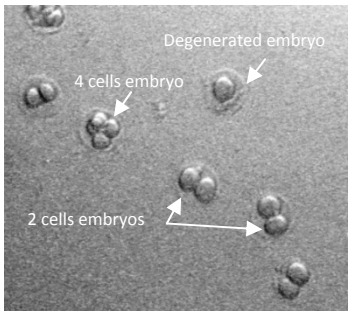

Figure 1. Embryos recovered at 24 hours after the vaginal plug

The 2 cells embryos recovered from the females were cultivated in M16 media. The incubation was performed in incubator at 37°C, in 5% CO_2 in air. The development of the embryos was evaluated at 24, 48 and 72 hours interval.

The results obtained at 24 hours are presented in table 2.

Table 2.

Results at 24 hours of cultivation in M16 media

Recovery media	Total N	Developmental stages of the embryos									
		Degenerated		2 cells		4 cells		8 cells		16 cells (early morula)	
		n	%	n	%	n	%	n	%	n	%
PBS 0,4% BSA	56	3	5.3[a]	16	28.6[A]	7	12.5[a]	26	46.4[a]	4	7.2[a]
M2	67	4	6[a]	2	3[a]	12	17.9[a]	38	56.7[a]	11	16.4[a]

test χ^2, $A - a$ $p < 0,001$, $a - a$ $p > 0,05$

From the data presented in table 1, it can be seen that at 24 hours from the 56 embryos recovered in PBS, 3 (5.3%) were degenerated, 16 (28,6%) were in 2 cells stage, 7 (12,5%) were in 4 cells stage, 26 (46.4%) were in 8 cells stage and 4 (7.2%) were in 16 cells stage.

In respect to the embryos recovered in M2 media from the 67 embryos, 4 (6%) were degenerated, 2 (3%) were in 2 cells stage, 12 (17.9%) were in 4 cells stage, 38 (56.7%) were in 8 cells stage and 11 (16.4%) were in early morula stage.

It can be noticed that the proportion of embryos that remained in 2 cells developmental stage (blocked in development) is higher at the embryos recovered in PBS 0.4% BSA (28,6%) compared to the embryos recovered in M2 media (3%). The difference is statistically very significant ($p<0,001$). Also the proportion of embryos that were in 8 cells stage is lower (46.4%) at embryos recovered in PBS 0.4 % BSA compared to the embryos recovered in M2 media (56.7%) but the differences were not significant statistically ($p>0,05$).

The results obtained at 48 cultivation hours are presented in table 3.

Table 3

Results after 48 hours of cultivation in M16 media

Recovery media	Total	Developmental stages of the embryos									
		Degenerated		8 cells		16 cells (early morula)		Compacted morula		Early blastocyst	
		n	%	n	%	n	%	n	%	n	%
PBS 0,4% BSA	56	24	42.8[b]	5	8.9[a]	11	19.6[a]	12	21.4[a]	4	7.1[a]
M2	67	16	23.9[a]	7	10.4[a]	17	25.4[a]	22	32.8[a]	5	7.5[a]

test χ^2, $a - a$ $p < 0,05$, $a - a$ $p > 0,05$

From the data in the table it can be seen that from the 56 recovered in PBS with 0,4% BSA embryos cultivated in M16 at 48 hours 24 (42.8%) embryos were degenerated, 5 (8.9%) embryos were in 8 cell stage, 11 (19.6%)embryos were in early morula stage, 12 (21.4%) embryos were in compacted morula stage and 4 (7.1%) embryos were in early blastocyst stage.

For the 67 embryos recovered with M2 and embryos cultivated in M16 at 48 hours 16 (23.8%) embryos were degenerated, 7 (10.4%) embryos were in 8 cell stage, 17 (25.4%) embryos were in early morula stage, 22 (32.8%) embryos were in compacted morula stage and 5 (7.5%) embryos were in early blastocyst stage.

After 48 hours of cultivation the proportion of degenerated embryos was significantly higher ($p<0.05$) at the embryos recovered with PBS and 0.4% BSA (42.8) compared with the embryos recovered with M2 media. The percent of embryos in compacted morula stage did not differed statistically between the two lots ($p>0.05$).

The results obtained at 72 cultivation hours are presented in table 4.

Table 4

Results after 72 hours of cultivation in M16 media

Recovery media	Total	Developmental stages of the embryos							
		Degenerated		Blastocyst		Expanded blastocyst		Hached	
		n	%	n	%	n	%	n	%
PBS 0,4% BSA	56	26	46.4[b]	8	14.3[a]	13	23.2[c]	9	16.1[A]
M2	67	16	23.9[a]	9	13.5[a]	6	8.9[a]	36	53.7[a]

test χ^2 | a – a $p < 0,05$, A – b $p < 0,01$ A – a $p < 0,001$;a-a>0,05

From table 4 it can be seen that at 72 hours form recovery from 56 embryos recovered with PBS 0.4% BSA 9 (16.1%) hatched. The proportion of degenerated embryos was 46,4% (n=26). The remaining embryos were in blastocyst stage (n=8, 14.3%) and (n=13, 23,2%) expanded blastocyst.

In respect to the embryos recovered in M2 media from the 67 embryos cultivated 36 hatched (53.7%). 16 (23.9 %) embryos were degenerated, 9 (13,5 %) were in blastocyst stage and 6 (8.9%) were in expanded blastocyst stage.

The proportion of hatched blastocyst was higher at the embryos recovered with M2 (53.7%) compared with the embryos recovered with PBS 0.4% BSA. The difference is statistically very significant ($p<0.001$). The expanded blastocyst proportion in embryos recovered with PBS was distinctly significantly higher (23.2) compared with the embryos recovered in M2 media (8.9) ($p<0.05$). Degenerated embryos proportion was higher after 72 hours of cultivation at embryos recovered with PBS (46.4) compared with embryos recovered with M2 (23.9) ($p<0.01$).

Sodium bicarbonate and lactate present in M2 but not in PBS medium in involved in the regulation of the pH via transporter proteins such as $HCO3^-/Cl^-$ exchangers and H+/monocarboxylate cotransporters (Y. Zhao, 1996). This may be the cause of the differences between the two hatching rates registered.

Conclusions

1. The majority (78.3%) of the embryos recovered at 24 hours after the vaginal plug were in 2 cell stage;
2. At 24 hours of cultivation the proportion of embryos that remain in 2 cell stage is higher (28.6%) at embryos that were recovered in PBS 0.4 BSA compared with the embryos recovered in M2 media (3%). The difference is very significant statistically ($p<0,001$);
3. At 48 hours of cultivation the proportion of degenerated embryos was significantly higher ($p<0.05$) at the embryos recovered with PBS and 0.4% BSA (42.8) compared with the embryos recovered with M2 media;
4. At 72 hours of cultivation the proportion of hatched blastocyst was higher at the embryos recovered with M2 (53.7%) compared with the embryos recovered with PBS 0.4% BSA. The difference is statistically very significant ($p<0.001$);
5. After 72 cultivation hours the proportion of degenerated embryos was higher after 72 hours of cultivation at embryos recovered with PBS (46.4) compared with embryos recovered with M2 (23.9)($p<0.01$);
6. Embryos recovered in M2 media have a higher *in vitro* developmental capacity compared with the embryos recovered in PBS media supplemented with 0,4% BSA, possibly because of the sodium bicarbonate and lactate used in M2 media for pH regulation.

Acknowledgement: This work was supported by PN II-IDEI project, contract number 2515/2008.

References

1. Edwards L.J., D.A. Williams, D.K. Gardner, 1998, Intracellular pH of the preimplantation mouse embryo: effects of extracellular pH and weak acids, *Mol. Reprod. Dev.* **50,** 434–442.
2. D. Lagadic-Gossmann, L. Huc, V. Lecureur, 2004, Alterations of intracellular pH homeostasis in apoptosis: origins and roles, *Cell Death Differ.* **11,** 953–961.
3. Toru Takeo, Takehito Kaneko, Yukie Haruguchi Kiyoko Fukumoto, Hiromi Machida, Mika Koga,Yoshiko Nakagawa, Yumi Takeshita, Toyokazu Matsuguma, Shuuji Tsuchiyama, Norihiko Shimizu, Takanori Hasegawa, Motohito Goto, Hitoshi Miyachi, Masayuki Anzai, Ena Nakatsukasa, Koji Nomaru, Naomi Nakagata. 2009, Birth of mice from vitrified/warmed 2-cell embryos transported at a cold temperature, *Cryobiology*, www.elsevier.com/locate/ycryo
4. Y. Zhao, J.M. Baltz, 1996. Bicarbonate/chloride exchange and intracellular pH throughout preimplantation mouse embryo development, *Am. J. Physiol*. **271** C1512–C1520.