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STUDIES REGARDING THE VIABILITY OF MOUSE EMBRYOS RECOVERED IN EARLY DEVELOPMENTAL STAGES

STUDII PRIVIND VIABILITATEA EMBRIONILOR DE ŞOARECE RECOLTAŢI ÎN STADII TIMPURII DE DEZVOLTARE

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In order to evaluate the embryo viability we tested 2 cultivation media in three combinations (M16, Nutrient mixture F- 12 HAM supplemented with BSA and Nutrient mixture F- 12 HAM without BSA). In the third day of cultivation the embryos viability was assessed by morphological criteria and randomly by staining with fluorescents dyes (Fluorescein diacetate and Propidium iodide). It was observed that both media used for in vitro cultivation assured embryo development up to the expanded blastocyst stage (21.33% for M 16 and 4.34% respectively), exception the Nutrient mixture F- 12 HAM without BSA in which after 3 days cultivation all embryos were degenerated. The M16 medium assured the embryos development up to the hatched blastocyst stage (5.33%). None of the media supported the development of embryos under 8 cell stages and early blockage in stage of 2 cells could not be prevented.

Key words: embryo, viability, culture medium, FAD, PI

Introduction

The micro-drops technique used for embryo cultivation was first introduced in the year 1963 by the R. Brinster and is still widely used. Since then, the composition of cultivation media was dramatically changed by the time we succeed to understand better the embryos necessities and lacks of the artificial environments. Efforts to culture in vitro embryos met various successes; even though is extremely difficult to culture them past to the four-cell stage. Later attempts to overcome this "in vitro developmental block" included culturing embryos in coculture, supplementation with complex biological fluids and various modifications of simple culture media. Although the achievements made early stages of embryonic development feasible in vitro, the adequate culture conditions for the preimplantation embryo still have to be improved. There are numerous data showing that embryonic development in vitro is retarded and results in fewer cell numbers compared to those in embryos developed in vivo, which is true. It has not

been determined, if extent this lower cell number compromises the further development of the cultured embryos.

Materials and Methods

The embryos were recovered from superovulated mice females 48 hours post coitus. The superovulation treatment was made administrating 5 UI/ml PMSG injection fallowed at 48 hours by an other 5 UI/ml hCG injection. The culture media used (M2 culture medium cat. no. M7167; M16 culture medium cat. no. M7292; Nutrient mixture F 12, cat. no. N 66 58 provided by Sigma-Aldrich,) were supplemented with 100 µl Pen-Stept/10 ml medium and incubated for 3 hours prior usage. The cultivations conditions were: 37°C, 5% CO_2 in atmosphere. For staining with fluorochrome were prepared 2 stock solutions:

- 5 mg Fluoresceind diacetate (FDA) dissolved in 1 ml acetone;
- 1 mg Propidium iodide (PI) dissolved in 1 ml M 2 medium;

In order to stain the embryos were prepared 2 working solutions:

- 50 µl stock solutions FDA dissolved in 1 ml M2 medium
- 50 µl stock solutions PI dissolved in 1 ml M2 medium.

The embryos were than randomly introduced in 100 µl and incubated for 10 minutes and than visualized at a fluorescence microscope at 490-518 nm for FDA and 515-560 nm for PI.

Results and Discussions

The total number of embryos recovered and the stages of the preimplantational embryos development are presented in table 1.

Table 1

Developmental stages of the recovered embryos

Recovered embryos	8 cells		4 cells		2 cells		degenerated	
n	n	%	n	%	n	%	n	%
202	58	28.71	36	17.82	27	9.40	81	76.25

In table 2 are presented the development stages at 24 hour of cultivation. As can be seen from the data's presented after 24 hours of cultivation 13.04% of the embryos cultivated in F-12 Ham + BSA and 5.33% of the embryos cultivated in M16 medium reached to the incipient blastocyst stage. 29.33% of the embryos cultivated in M 16 medium and 36.2% of the embryos cultivated in Nutrient mixture F-12 Ham without BSA were retarded (in 2 and 3 cells embryo stages). All mediums supported embryos development up to the morula stages. None of the embryos recovered under 8 cells developmental stages past over the stage that have been recovered.

Table 2

The developmental stages at 24 hours of cultivation

Culture medium	Embryos cultivated	Incipient blastocyst		Morula		Retarded		Degenerated	
	n	n	%	n	%	n	%	n	%
M 16	75	4	5.33	17	22.66	22	29.33	32	42.66
F-12 HAM+BSA	69	9	13.04	18	26.08	20	28.98	22	31.9
F-12 HAM	58	-	-	10	17.24	21	36.2	27	46.55

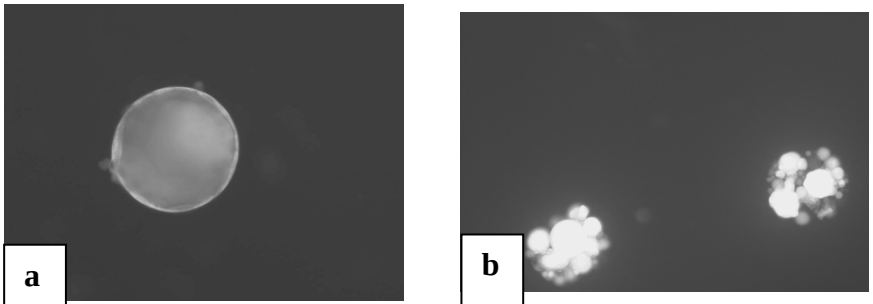
The M 16 and F-12 Ham+BSA supported the embryo development up to the expanded blastocyst stages (21.33% for M 16 and 4.34% for the F-12 Ham+BSA medium) and only the M 16 medium assured the blastocyst hatching (5.33%), (Table 3). The embryos cultivated in F-12 Ham without BSA were all degenerated.

Table 3

The developmental stages at 48 hours of cultivation

Culture medium	Embryos cultivated	Hatched blastocyst		Expandat blastocyst		Blastocyst		Blastocist incipient		Morula		Retarded		Degenerated	
	n	n	%	n	%	n	%	n	%	n	%	n	%		%
M 16	75	4	5.33	16	21.33	-	-	-	-	1	1.33	13	17.33	41	54.68
F-12 HAM+BSA	69			3	4.34	5	7.24	1	1.44	3	4.34	1	1.44	56	81.96
F-12 HAM	58	All degenerated													

After 48 hour of cultivation the embryos were randomly selected in order to be evaluated by staining with fluorescent dyes (Fluorescein diacetate and Propidium iodide – Figure 1 a, and b).



Figur Viable embryo stained with FAD egenerated embryos stained with FDA and PI

The viable embryos stained with FDA emitted green fluoresces while being examined with a fluorescent microscope at 494-518 nm and the nonviable embryos stained with PI emitted red fluorescence while being examined at 515-560 nm. Fallowing staining we could notes that some of the embryos retarded in development were still manifesting, partially, enzymatic activity within the balstomers even if their development was blocked during the in vitro cultivation.

Conclusions

1. The M16 and F-12 Ham + BSA medium assured embryo development up to the expanded blastocyst stage (21.33% for M 16 and 4.34% respectively), exception the Nutrient mixture F- 12 HAM without BSA in which after 3 days cultivation all embryos were degenerated.
2. 5.33% of the embryos cultivated in the M16 medium reached to the hatched blastocyst stage. None of the media supported the development of embryos under 8 cell stages and early blockage in stage of 2 cells could not be prevented.
3. The embryos evaluated as degenerated manifested partially enzymatic activity while being exposed in fluorescence after staining with FDA and PI.

Bibliography

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