Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 4, 2011, pp. 723-726
©2011 Vasile Goldis University Press (www.studiauniversitatis.ro)

MEDIUMS QUALITY AND THEIR ABILITY TO SUPPORT DEVELOPING OF PREIMPLANTED MOUSE EMBRYOS

Svetlana KOGĂLNICEANU-ODOBESCU[1], Ioana LĂNCRĂJAN[1], Doru Ardelean[1], Ioan VINTILĂ[2]
[1]„Vasile Goldiş" Western University of Arad, Faculty of Life Sciences, Romania
[2]University of Agricultural Sciences and Veterinary Medicine, Timişoara, Romania

ABSTRACT
Embrionary development begins with the fertilization, after the formation of zygote. Our aim was the study some factors (temperature, media, atmosphere) that can influence the development of preimplantation embryo by different species and humans. The influence of culture medium in which the embryos were grown was under research regarding the transfer of embryos from *in vivo* medium to *in vitro* culture media. We investigated methods for developing culture media and determining requirements for development *in vitro*.
Inactived rat serum and addition of protein (FCS), on defined culture medium TCM 199, rise the efficiency of growing method, and exhibit higher ability to support blastocysts developing until eclosing from zona prellucida as the correspondingly defined media.
Keywords: embryos, media, preimplantation, fertilization, in vitro culture.
Abbreviations: FCS - fetal calf serum, ICM - inner cell mass, ROS - reactive oxygen species, β-mercaptoethanol (β-MEC).

INTRODUCTION

Embrionary development begins at a time with the fertilization, which has as result formation of the zygote. Different cells division, cells differentiation take place, which always adds something structural and functional attributes to the product of conception. The normal development of the embryo requires a specific environment. Defined culture mediums create a necessary condition for "in vitro" development of embryos. The influence of different culture media on the development of preimplantational embryos differs with species (Sananmuang, 2010; Kataria et al., 2010).

The production of embryos in the laboratory follows superovulation, harvesting of ovocytes, harvesting of sperm cells, co-growing of the female seminal material and of sperm cells in the purpose of fecundation and obtaining zygote is still pretty expensive. Because of this we thought about studying deeper each step of work, each factor (temperature, environment, atmosphere) which can influence, in a way or another, the further development of preimplanted embryos at different species of animals, as well as on the humans. That's why the aim of our work was to see how different environments of culture were influencing the further development of preimplanted mouse embryos, which were developing "in vivo" until the time of "in vitro" cultivation.

MATERIALS AND METHODS

The biological material used for our experiments were consisted in white female mice, from which were harvested embryos on certain preimplantational stages.

Embryos were harvested from the uterine horns 96 hours after mating, when they were found in the compact morula and blastocyst.

After harvesting the growing of embryos were performed in different culture mediums

Table 1

Standard mediums for growing of mouse preimplanted embryos (pH 7,4)

Components	Whitten (w-i971)	Brinster (BMOC-2)	Donahue (DONH)	Phosphate (pB-1)
NaCl	514mg	554mg	696mg	781mg
KCl	36mg	36mg	35mg	20mg
KH_2PO_4	16mg	16mg	16mg	19mg
$MgSO_4 \times H_2O$	29mg	29mg	29mg	-
$NaHCO_3$	190mg	211mg	210mg	9,8mg
Na-piruvat	3,5mg	2,8mg	3,5mg	3,6mg
Ca-lactat x $5H_2O$	53mg	-	-	-
Na-lactat	242mg	225mg	-	-
Glucose	100mg	100mg	-	100mg
$CaCL_2$	-	19mg	19mg	10mg
$NaHPO_4 \times 2H_2O$	-	-	-	143mg

Corespondence: *Svetlana Kogălniceanu-Odobescu, „Vasile Goldiş" Western University of Arad, Faculty of Life Sciences, email: sos_kg@yahoo.com*

$MgCl_2$ x $6H_2O$	-	-	-	10mg
Penicylin	8mg	10mg	6,3mg	8mg
Streptomycin	5mg	5mg	5mg	5mg
Phenol red	1mg	1mg	1mg	1mg
BSA	300mg	100mg	100mg	395mg
Miliosmoli	295	320	309	310

The following culture mediums were used: TCM-199 unsupplemented medium, TCM-199 supplemented medium with 10% FCS (bovine fetal serum), the inactive mice blood serum and HAM'S F-10 medium.

The cultivated blastocysts in the TCM-199 unsupplemented medium were incubated in conditions of carbon dioxide deficiency. The harvested embryos were grown in different culture mediums for 24 hours and then the analyses of embryos development in the given conditions of each medium were made.

The hatching moment of the embryo from the *zona pellucida* was considered to be the final aim of the „in vitro" cultivated embryos in every of the mediums we used.

The influence of each used culture medium was appreciated after 24 hours, regarding the normal development and hatching of blastocysts in *zona pellucida*.

RESULTS AND DISCUSSIONS

208 embryos were harvested in an advanced stadium of blastocyst which was assigned in four different experimental ways: TCM 199 (V1), TCM supplemented with 10% FCS (fetal calf serum) V2, inactivated rat serum (V3) and HAM'S F-10(V4) medium. The hatching rate of blastocysts after 24 hours is represented on the table 2.

Table 2

Eclosion frequency of advanced blastocysts growth on different mediums

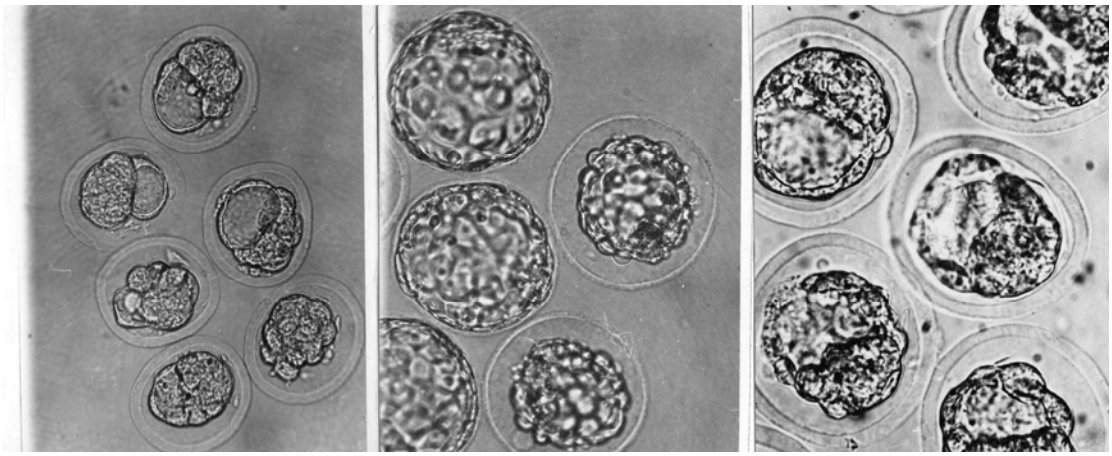
Culture medium	Number of cultured embryos	Number of embryos develloped afterwads	Percent % of eclosion
TCM 199	32	-	0 %
TCM 199 +10 % FCS	64	51	79,7 %
Rat inactivated serm	60	30	50 %
HAM'S F-10	52	-	0 %

Our data shows, as resulted from the table 2, different eclosion frecvency of blastocyst, absolutelly variable, depending on culture medium.

In case V1, by growing of embryos on simple TCM 199 medium, they survived only some hours.

Quickly, the trophoblast cells begin to agglomerate around the blastocyst, in form of a cells agglomeration, around of them beiyng located the fluid cavity of blastocyst surrounded by *zona prellucida*.

Blastocyts before described represent embryos that are not more alive.



(A) (B) (C)

Fig.1. Development of embryos in TCM199 culture medium embryos in stage morula, (B) embryos in stage compact morula, (C) embryos in stage inchoate and medium blastocyst

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 4, 2011, pp. 723-726
©2011 Vasile Goldis University Press (www.studiauniversitatis.ro)

Compaction is the first event of morphogenic and cellular differentiation. The most significant event occurring at compaction is the emergence of 2 distinct cell populations: the blastomeres remaining in contact with the outside are destined to form the trophectodermal lineage while the blastomeres inside the embryo are destined to form the ICM (inner cell mass) (Sakkas).

In case of V2, by 10% FCS supplemented TCM199 culture medium, the blastocysts has followed their natural development reaching the eclosed blastocyts stage.

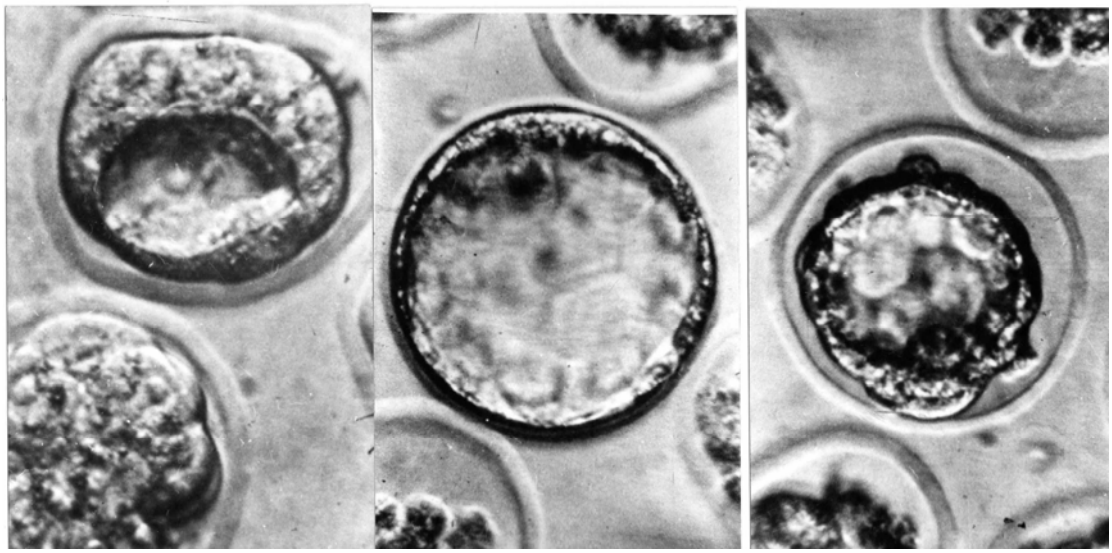
As showed on table 2, eclosion rate in case of V2 was 79.7%. The result certified that the embryos on the blastocyt stage needs together with the composition of defined medium additionally proteins. Supplementation of medium with proteins, in this case FCS, made possible the development of preimplanted embryos from blastocyst to the last preimplantational stage, respectively eclosed blastocyst.

In the case of V3, the chosen culture medium was inactivated rat serum.

Even if this medium is characterized by large amount of proteins, the frequency of eclosion was lower as in case of V2. Probably, in the inactivated rat serum some growing factors are missing, or they are present in insufficient amounts.

I case of V4 cultured medium was HAM'S F-10. None from the 52 cultured embryos could develop. This was surprising because the human embryos growth on this medium exhibit a well development and reached the expanded blastocyst stage at 80-88 % ratio.

The influence of culture media regarding 4 components (NaCl, pyruvate, KH_2PO_4 and glucose) (Lawitts, 1991), medium oxygenation (Vijayakumar, 1987) and antioxidant supplement (Hussein, 2009), embryos from different species: cat (Sananmuang, 2010), rats (Heindryckx, 2001), mouse (Abassali, 2005) were studied before.



A B C

Fig.2. (A) and (B) embryos developed TCM199 culture medium supplemented with 10% FCS; (C) embryos growth on HAM S F-10 medium

Supplementation of exogenous antioxidants such as β-mercaptoethanol (β-MEC) plays a critical role in increasing the resistance of embryos to reactive oxygen species (ROS) especially when added throughout the culture period. However, antioxidant inclusion during in vitro embryo development is not sufficient for ROS detoxification during the critical period of post-warming embryo culture. The exact mechanism by which β-MEC and other antioxidants protect the post implantation embryo needs to be elucidated.

The observed positive effect of supplemented with 10% FCS culture mediums in our experiments could be explained partially because of antioxidant effect of serum through included naturally lipids, that became substrate for ROS.

CONCLUSIONS

The analysis of results shows a high variety of eclosion rate when embryos are growth on all chosen culture mediums.

The eclosion frequency of embryos indicates the media abilities of to promote the development in vitro.

Due to the fact that the percent of eclosion in case V1 and V4 was zero, we conclude that the corresponding media are not efficiently on cultivation of mouse blastocysts. Consequently, the influence of TCM 199

©2011 Vasile Goldis University Press (www.studiauniversitatis.ro)

and HAM' S F10 culture mediums on developing until eclosion of blastocyst can be characterised as negative.

The results evidence 50 % and respectively 79,7 % eclosed blastocyst in inactive rat serum and respectively on medium TCM 199 supplemented with 10 % FCS.

These results enable to conclude that inactive rat serum on the first case and addition of FCS, a protein source, on defined culture medium TCM 199, rise the efficiency of growing method on defined culture mediums, with positive impact on blastocysts developing until ecosingfrom zona prellucida.

REFERENCES

Abbasali Karimpor Malekshah, Ph.D., Amir Esmailnejad Moghaddam, The effect of culture medium volume on in vitro development of mouse embryos, Iranian Journal of Reproductive Medicine Vol.3. No.2 pp:79-82, 2005

Adams CE, The relationship between mother and embryo during the preinplantation period with particular reference to fertility and fitness of the offspring in: Experimental Embriology and Teratology (D.H.M. Woollam and J.M. Morris, eds.)Elek Sci. London, pp.1-15,1974

Adams CE, Hay MF, Lutwak-Mannk, The actions of various agents upon the rabit embryo. J. Rbryol. Exp. Morphol., 9, 468-491, 1961

Amel SA, Cell death in mouse embryos preimplantation. Differentiation 38, 127-131, 1989

Byskov AG, Hoyer PE, Embryology of mammalian gonads and duets. In Knobil E., Neil JD (eds), The Physiology of Reproduction, New York: Raven Press, 487-540, 1994

Bhorn Heindryck X, Rybouchkin A, van der Elst J Dhont M, Effect of Culture Media on In Vitro Development of Cloned Mouse Embryos, Clonning, Mary Ann Liebert, Inc., Volume 3, Number 2, 2001

Brinster RL, A method for in vitro cultivation of mouse ova from two cell to blastocyst. Exp. Cell Res., nr. 32, pp. 205-208, 1963

Collas P, Robl M, Factors affecting the efficiency of nuclear transplantation in the rabbit embryo. Bio Reprod. 43:877-884, 1990

Kataria N., Kataria A. K., Pandey N., Gupta P., Serum biomarkers of physiological defense against reactive oxygen species during environmental stress in Indian dromedaries, HVM Bioflux, 2(2), pp.55-60, 2010.

Longo FJ, Fertilization Champan and Hall Co., London – New York, 1987.

Dobrinsky J.R, Johnson L.A., Rath D, Development of a Culture Medium (BECM-3) for Porcine Embryos: Effects of Bovine Serum Albumin and Fetal Bovine Serum on Embryo Development, Biololgy of Reproduction 55, 1069-1074, 1996

Hosseini, S. M. Forouzanfar M., Hajian M., Asgari V., Abedi, L. P. , Hosseini, S. Ostadhosseini, F. Moulavi, M. Safahani Langrroodi, H. Sadeghi, H. Bahramian, Sh. Eghbalsaied, Mohammad H. Nasr-Esfahani

Antioxidant supplementation of culture medium during embryo development

and/or after vitrification-warming; which is the most important?

J Assist Reprod Genet 26:355–364, 2009

Lawitts JA, Biggers JD, Optimization of mouse embryo culture media using simplex methods, J Reprod Fertil. Mar; 91(2):543-56, 1991

Rogers BJ, Bentwood BJ, Capacitation acrosome reaction and fertilization, Zaneveld L.J.D., Chatterton R.T. (eds.), Biochemistry of mammalian reproduction, 203-230, New York Wiley, 1982.

Rieger D, Relationships between energy metabolism and development of early mammalian embrios. Theriogenology, vol. 37, nr. 1, pp.75-93, 1992

Vlaic A, Inginerie genetică. Editura Promedia Plus, Cluj-Napoca, 1997.

Sananmuang T, Tharasanit T, Techkumphu M, The effects of culture media and embryo densitiy of cat embryos, Proc 9th CU VetSci Ann Con, pp. 15, 2010

Vijayakumar R., Simoni J., Ndubisi B., Deleon F.and Heine W., Mouse Embryo Growth in Different Culture Media: Selection of a Medium for Quality Control Cross-Testing of Human in Vitro Fertilization Conditions Vol. 19, No. 2 , Pages 149-158, 1987

©2011 Vasile Goldis University Press (www.studiauniversitatis.ro)