The Seoul Journal of Medicine
Vol. 30, No. 3:143-148, September 1989

In Vitro Cultivation and Transplantation of Mouse Embryos: Comparative Analysis of Effects of Bovine Serum Albumin and Sodium Lactate

Sang Ho Baik, Goo Bo Jeong * and Douk Ho Hwang

Department of Anatomy, College of Medicine, Seoul National University, Seoul 110-460, Korea

= Abstract =Two-cell embryos were recovered from ICR, Balb/c, and F1 hybrid (CBA × C57Bl/6) females, and cultured in vitro in the Ham's F-10 medium supplemented with 0.1 % BSA, 20 mM Na-lactate, or 10 mM HEPES. With F1 hybrid embryos, there were significantly higher development in Ham's F-10 medium supplemented with 0.1% BSA (79.1%) and lower development in that supplemented with 10 mM HEPES. Significant differences between strains were seen also, and preimplantation development was significantly better with the embryos of the F1 hybrid strain and was lower with the embryos of the Balb/c strain. It was concluded that BSA and Na-lactate are the important factors affecting preimplantation development, and embryos of the F1 hybrid strain are less sensitive to culture conditions.

After transplantation of blastocysts cultured in vitro, the pregnancy rate of recipient mice and implantation rate of transferred embryos were 60% and 32%, respectively.

Key words: ***Zygote, Culture, Embryo transfer, Mice, BSA, Sodium lactate***

INTRODUCTION

Since the early efforts by Hammond (1949) to culture mouse ova in a medium composed of a simple salt solution supplemented with glucose and egg white resulted in survival of eight-cell embryos, scientific progress during the last four decades have led the present capability of nurturing the embryonic development from zygote to blastocyst stages *in vitro* for several mammalian species (Whitten and Biggers, 1968; Maurer *et al.*, 1971; Kane, 1972; Tervit *et al.*, 1972; Whittingham, 1975) and humans (Edwards *et al.* 1970).

Although the mechanism for such improvement is not completely clear, crystalline bovine serum albumin was reported to support development of two-cell to morula-stage rabbit embryos (Brinster, 1970), and the chemically defined medium when supplemented with 0.1 or 0.2% BSA was superior to several complex tissue culture media in maintaining viability of sheep embryos *in vitro* (Wright *et al.*, 1976). There is an enhancement of two-cell embryo to blastocyst development when optimal concentrations of pyruvate and lactate were combined (Brinster, 1965). Slightly hypotonic conditions may favor early embryonic development *in vitro*, and the optimal osmolarity for development of two-cell mouse embryos was found to be 276

Received 18/7/89; revised 21/8/89; accepted 23/8/29

*Present address: Department of Anatomy, College of Medicine, Chungbuk National University, Cheong Ju 360-763, Korea

**This study was supported in part by SNU Endowment Fund for Medical Research China Medical Board RC-83-1 (1983)

mOsm (Brinster, 1965).

In addition, it is now clear from experiments with mouse embryos that during preimplantation development there is a gradual change in energy substrate requirements (Brinster, 1973).

Embryos apparently require additional as yet unknown factors that ensure their continued development after *in vitro* culture (Mills *et al.*, 1973), but defined culture media apparently lack important constituents that ensure continued *in vivo* development of blastocysts even though the embryos can be cultured to this stage *in vitro*. therefore more complex media are necessary for cleavage (Brackett *et al.*, 1980).

In the mouse, development to late morula or early blastocyst is a prerequisite to subsequent normal uterine development, and to advance knowledge in embryo culture, several factors must be born in mind. But despite many reports about the embryo culture in mice, the culture rate is variable depend on the strains, medium constituents, and culture condition, and the success rate of implantation after embryo transfer is low yet.

In this study, we have attempted to elucidate some important factors that will raise up the present rate of success in the culture of mouse embryos and in the implantation of transferred embryos.

MATERIALS AND METHODS

Animals

Inbred Balb/c and C57Bl/6 strain mice obtained from the Korea Research Institute of Chemical Technology (Daedeog, Chungnam) and Inbred CBA, ICR strain mice obtained from Seoul National University (Seoul) were used. Animals were kept at constant temperature (24°C) and automatically regulated exposure to light from 5 am to 7 pm. The animals were fed standard lab chow (Jeil Co.) and water *ad libitum*. Virgin females were mated overnight with fertile males, and 8-week offsprings were used in these experiments.

Embryos were obtained from Balb/c, ICR, and F1 hybrid (CBA ♂ X C57Bl/6 ♀) mice. To induce superovulation, PMSG (Sigma Co.) and hCG (Sigma Co.) were injected 44-47 hrs apart respectively. Immediately after injection of hCG, one female was caged with each male, and mating was noted by the presence of a copulatory plug the next morning. Two-cell embryos were obtained from mated females 46 hours after the injection of hCG, and were cultured in Ham's F-10 medium (Gibco lab.) and those supplemented with other components.

Pseudopregnant foster mothers were made by mating with vasectomized ICR males, cultured blastocysts of F1 hybrid mice were transferred to pseudopregnant ICR females.

Embryo Culture

Two-cell embryos were flushed from the uterine tubes of mated females with Ham's F-10 medium (Gibco lab.) supplemented with 10 mM Hepes buffer. Flushed two-cell embryos were pooled and twenty embryos were cultured in each drop of culture dishs (Costar plastics, # 3035) containing 4 drops of culture medium under a layer of paraffin oil (Fluka). The embryos were incubated at 37°C in an humidified atmosphere of 5% CO_2 in air.

The osmolarity of the medium used was 270-310 mOsm. To compare culture rate of two-cell embryos in the same group Ham's F-10 medium and those supplemented with 0.1 % BSA, 20 mM Na-lactate, or 10 mM HEPES buffer were used respectively. And Ham's F-10 medium supplemented with 0.1% BSA was used for comparing the rate among strains.

Transplantation

The embryos were examined for blastulation under a inverted microscope (X100, Labovert, Leitz) at 12-hr intervals for 3 days. After 72 hr-cultivation, the embryos were evaluated by means of inverted microscopy. Cultured blastocysts of F1 hybrids were transferred to the left uterine horn of pseudopregnant ICR females. The methods of Hogan *et al.* (1986) was used in transplanting the blastocysts into the uterine horns. The percentage of two cell embryos developing to blastocyst and of blastocysts growing further to fetuses after the transfer was

calculated.

RESULTS

The quantitative evaluation of ova cultured *in vitro* was carried out in two experiments. In the first, 2-cell embryos from ICR females were cultivated 72 hours in four kinds of media to find factors affecting the culture rate. From the results presented in Table 1, it can be seen that 72 hours after the incubation in the basic Ham's F-10 medium, 72.2% of the 2-cell embryos was developed into blastocysts. The percentage of blastocysts developed in cultivation decreased markedly when 10 mM HEPES was added to the Ham's F-10. The most consistent feature of these embryos was the prolonged time required for completion of the 4-cell or more. This delay was then maintained throughout subsequent development. Many of the embryos were capable of developing to the eight-cell stage, but continued development often ceased. But there was no single point at which all embryos arrested.

Table 1. Development of embryos *in vitro* after induced ovulation

Medium	Culture rate (%)
Ham's F-10	72.2 (260/360)
Ham's F-10 + 0.1% BSA	75.1 (215/298)
Ham's F-10 + Na-lactate	74.0 (140/200)
Ham's F-10 + 10 mM Hepes	20.0 (40/200)

The best medium to development of embryos was that containing bovine serum albumin, and there was no striking difference between the effectiveness of 0.1% BSA and 20 mM Na-lactate in development *in vitro*.

To compare the difference of embryonic development among strains, embryos from three kinds of mice (ICR, Balb/c, and F1 hybrid) were used in cultivation. The results are given in Table 2. The difference among strains was great. Developmental potential of F1 hybrid embryos was best and that of Balb/c embryos was lowest.

Table 2. Preimplantational development of embryos according to the strains of mice

Strain	Culture rate (%)
ICR	72.1 (215/298)
Balb/c	55.0 (132/240)
F1 hybrid	79.1 (497/628)

The mean cultivation rate of F1 hybrid embryos was slightly higher (79.1%) than that of ICR embryos (72.1%), but the difference between Balb/c mice and the other strains was great. Indeed, when embryos of the F1 hybrids were cultured in a medium supplemented with 0.1% BSA, the developmental rate was significantly higher than in any other medium conditions of ICR and Balb/c embryos.

In carrying out the transfer, 200 blastocysts of F1 hybrid mice were transplanted to 40 ICR females that had been mated with vasectomized males. From twenty-four pregnant females only sixty-four (32%) embryos were developed to term. And a sixty percent (24/40) pregnancy rate was observed. Genetic control was assured by the use of pigmented donors (F1, C57Bl/6 X CBA) and albinotic recipients (ICR).

DISSCUSSION

A considerable body of evidence shows that the cleaving ovum requires specific exogenous energy sources at different times of development (Biggers, Whittingham and Donahue, 1967), and these may be supplied by secretions of the uterine tubes. Our results, showing that bovine serum albumin and Na-lactate affect on the preimplantation development, are in full agreement with those of Brinster and Thomson (1966), and Brinster (1973). These authors, however, used chemically defined medium whereas in our experiments complex media were used. The developmental rate was lower in our experiments than that of other authors (Whitten and Biggers, 1968; Hoppe and Pitts, 1973), but the trend is similar. Although many factors such as glucose, pH, osmolarity, O_2 concentration etc. affect development of embryo, crystalline bovine albumin was reported to sup-

port development of two-cell to morula-stage rabbit embryos (Brinster, 1970), sheep embryos (Wright *et al.*, 1976), and mouse embryos (Hoppe and Pitts, 1973) etc. In our experiments albumin was an important factor for development of embryos. The mechanism for such improvement is not completely clear. But in our experiment, in spite of using complex media containing amino acids, medium supplemented with BSA increased the developmental rate. This indicates that BSA has an special effect in the development of embryos and that BSA acts independently of the use of amino acid. And 20 mM Na-lactate supported preimplantation development as well. Brinster and Thomson (1966) reported that mouse embryos use lactate or other components as energy source during preimplantational development. Despite the use of similar or more complex media, there is a difference in culture rate among authors. It appears that culture conditions and experimental skills such as manipulation of embryos etc. are another important factors in culture rate.

When HEPES is supplemented to the media the culture rate become lowest and development after 4-cell stage decrease markedly. Brinster (1972) has similarly reported deleterious effects in the use of some buffers (e.g. Tris, phosphate, HEPES). These experiments still indicate that the main effect of bicarbonate and CO_2 in equilibrium is not regulation of pH, but supply of a carbon source during development (Wales *et al.*, 1969; Graves and Biggers, 1970). Incubation of two-cell mouse embryos for only 2-4 hr in phosphate-buffered medium resulted in a reduction in their subsequent ability to develop into blastocysts (Quinn and Wales, 1973). This deleterious effect was especially stronger will the Balb/c strain (our unpublished data).

In the experiment to compare the difference among strains, the embryos of F1 hybrid females develop best. The percentage of random bred ICR embryos developing to blastocysts was comparatively less than that of the F1 hybrid. Many authors (Biggers, 1971; Whitten, 1971; Cross and Brinster, 1973) have reported that variable and limited success was obtained with random bred mice. In addition, in our experiment the percentage of Balb/c embryos developing to blastocysts was significantly less than random bred ICR and F1 mouse embryos, these results serve to demonstrate the sensitivity of the Balb/c two-cell mouse embryo to the same culture conditions and its complete dependency upon the environment for further development.

In carrying out the egg transfer, Table 3 provides a summary of the transfer of 200 embryos to 40 recipients. A moderate pregnancy rate (60%) and embryo survival rate (32%) were observed. The previously reported surgical method resulted in implantation rates of approximately 50% or less (Beatty, 1951; Tarkowski, 1959), but more recent investigation (Moler *et al.*, 1979) using nonsurgical egg transfer techniques have resulted in success rates approaching up to 60%. Some investigators (Marsk and Larrson, 1974) have attributed their success to minimizing the risk of losing embryos during transplantation. It is possible that crowding of transferred blastocysts into one uterine horn was responsible for poor success rates.

Table 3. Transfer of embryos

Number of recipients		Number of embryos	
Total	Pregnant	Total transfered	Developing to term
40	24 (60%)	200	64 (32 %)

REFERENCES

Beatty RA. Transplantation of mouse eggs. Nature. 1951, 168: 995

Biggers JD. New observations on the nutrition of the mammalian oocyte and the preimplantation embryo. In Blandau RJ (Ed) The Biology of the Blastocyst. University of Chicago Press, Chicago and London, 1971: pp. 319-325

Biggers JD, Whittingham DG, Donahue RP. The pattern of energy metabolism in the mouse oocyte and zygote. Proc. Natn. Acad. Sci. U.S.A. 1967, 58: 560-567

Brackett BG, Oh YK, Evans JF, Donawick WJ. Fertilization and early development of cow ova. Biol. Reprod. 1980, 23: 189-205

Brinster RL. Studies on the development of mouse embryos in vitro. IV. Interaction of energy sources. J. Reprod. Fertil. 1965, 158: 59-68

Brinster RL. Culture of two-cell rabbit embryos to morulae. J. Reprod. Fertil. 1970, 21: 17-22

Brinster RL. Cultivation of the mammalian egg. In Rothblat G and Cristofalo V (Ed). Growth, Nutrition, and Metabolism of cells in culture. Academic Press, Now York, 1972: pp. 251-286

Brinster RL. Nutrition and metabolism of the ovum, zygote, and blastocyst. In Grep RO and Astwood EA (Ed) Handbook of Physiology-Endocrinology II. American Physiological Society, Washington, D.C., 1973: pp. 165-185

Brinster RL, Thomson JL. Development of eight-cell mouse embryos in vitro. Exp. Cell Res. 1966, 42: 308-315

Cross PC, Brinster RL. The sensitivity of one-cell mouse embryos to pyruvate and lactate. Exp. Cell Res. 1973. 77: 57-62

Edwards RG. Steptoe P, Purdy JM. Fertilization and cleavage *in vitro* of preovulatory human oocytes. Nature (London). 1970, 227: 307-309

Graves CN, Biggers JD. Carbon dioxide fixation by mouse embryos prior to implantation. Science. 1970, 167: 1506-1508

Hammond J. Recovery and culture rate of tubal mouse ova. Nature (London). 1949, 163: 28-29

Hogan B. Costantini F, Lacy E. Embryo transfers. In Manipulating the mouse embryo. Cold Spring Harbor, New York, 1986: pp. 129-139

Hoppe PC, Pitts S. Fertilization *in vitro* and development of mouse ova. Biol. Reprod. 1973, 8: 420-426

Kane MT. Energy substrates on culture of single cell rabbit ova to blastocysts. Nature (London). 1972, 238: 468-469

Kane MT, Foote RH. Factors affecting blastocyst expansion of rabbit zygotes and young embryos in the defined media. Biol. Reprod. 1971, 4: 41-45

Marsk L, Larrson KS. A simple method for non-surgical blastocyst transfer in mice. J. Reprod. Fertil. 1974, 37: 393-398

Maurer RR, Whitener RH, Foote RH. Relationship of *in vivo* gamete aging and exogenous hormones to early embryo development in rabbits. Proc. soc. Exp. Biol. Med. 1969, 131: 882-885

Mills JA, Jeitles GGJ, Brackett BG. Embryo transfer following *in vitro* and *in vivo* fertilization of rabbit ova. Fertil. Steril. 1973, 24: 602-608

Moler TL, Donahue SE, Anderson GB. A simple technique for nonsurgical embryo transfer in mice. Animal Science. 1979, 29: 353-356

Mukherjee AB, Cohen MM. Development of normal mice by *in vitro* fertilization. Nature (London). 1970, 228: 472-473

Ogawa S, Satoh K, Hashimoto H. *In vitro* culture of rabbit ova from the single cell to the blastocyst stage. Nature (London). 1971, 233: 422-424

Quinn P, Wales RG. Growth and metabolism of preimplantation mouse embryos cultured in phosphate-buffered medium. J. Reprod. Fertil. 1973, 35: 289-294

Tarkowski AK. Experiments on the transplantation of ova in mice. Acta Theriol. 1959, 2: 251-267

Tervit HR, Whittingham DG, Rowson LEA. Successful culture in vitro of sheep and cattle ova. J. Reprod. Fertill. 1972, 30: 493-497

Wales RG, Quinn P, Murdoch RN. The fixation of carbon dioxide by the 8-cell mouse embryo. J. Reprod. Fertil. 1969, 20: 541-545

Whitten WK. Nutrient requirements for the culture of preimplantation embryos. In Raspe G (Ed.) Advances in the Biosciences. Pergamon Press, Oxford, 1971: pp. 129-141

Whitten WK, Biggers JD. Complete development in vitro of the preimplantation stages of the mouse in a simple chemically defined medium. J. Reprod. Fertil. 1968, 17: 399-401

Whittingham DG. Fertilization, early development and storage of mammalian ova. In Balls M and Wilds AE. (Ed.) The Early Development of Mammals. Cambridge University Press, London, 1975: pp. 1-24

Wright RW, Anderson GB, Cupps PT, Drost M, Bradford GE. *In vitro* culture of embryos from adult and prepubertal ewes. J. Anim. Sci. 1976, 42: 912-917

= 국문초록 =

생쥐 수정란의 시험관내 배양 및 이식 :
Bovine serum albumin 및 sodium lactate 효과의 비교분석

서울대학교 의과대학 해부학교실

백상호 · 정구보 · 황덕호

생쥐 난자의 배양 성적에 중요한 영향을 미치는 요인들을 비교 분석하기 위하여 Ham's F-10 배양액에 0.1% BSA를 추가한 군, 10 mM Na-lactate를 추가한 군 및 10 mM HEPES를 추가한 세 종류의 실험군을 설정하고 비교한 결과, 0.1% BSA를 추가한 군의 배양성적이 좋았다. 그리고 생쥐의 품종간의 비교를 위해 ICR, Balb/c, F1 hybrid (CBA ♀ × C57Bl/6 ♂)의 수정 난자를 배양한 결과 F1 hybrid의 난자에서 79.1%의 가장 좋은 배양 성적을 보였다. 이 결과는 생쥐의 배자 배양시 BSA와 Na-lactate가 중요한 인자로 작용하며 교잡종 생쥐의 난자가 같은 배양 조건에서 더 잘 자란다는 것을 보여 주는 것이다.

수정란 이식 실험에서는 F1 hybrid의 낭배를 40 마리의 가임산 ICR의 좌측 자궁각에 5개씩 이식한 결과 60%의 임신율과 32%의 이식 성공율을 얻었다.