A. Ü. Vet. Fak. Derg.
33 (3) :408 -415, 1986

A COMPARISON OF TWO CO_2 CHAMBERS ON THE DEVELOPMENT OF MOUSE EMBRYOS IN CULTURE

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Fare embriyolarının kültüre edilmesinde yararlanılan iki değişik CO_2 ortamı üzerinde çalışmalar

Özet: *Fare embriyolarının in vitro kültüre edilmelerinde vasattaki pH nın 7.2–7.4 de devamlılığını sağlamak için kullanılan değişik iki CO_2 ortamının karşılaştırması yapılmıştır.*

Bu ortalamalardan biri dört hareketli rafı ve bu raflardan herbirinde altı ufak petri alabilecek gözleri olan, tabanda gazın içeri girebileceği tavanda da dışarı çıkabileceği iki kapakcığı bulunan, etüv içine oturtulmuş, transparent bir kabindir. Embriyoların gelişmesi için uygun ortam sağlıyabilmek amacıyla kontrollu bir pompa yardımıyla kabin içine 24 saat devamlı % 5 CO_2'li hava verilmektedir. Bu gaz karışımının kabin içersindeki nemi arttırması amacıyla da su dolu bir silindirden geçirilmekte, bu aynı zamanda gaz akışının devamlılığını göstermesi açısından bir kontrol gibi kullanılmaktadır.

Diğer ortam ise kontrollu gaz giriş-çıkışları olan ticari bir anaerobik ortamdır. Kültürleri içeren bu jar'a (kavanoz) 10 dakika süreyle % 5 CO_2'li hava verilir daha sonra kapakcıkların sıkılmasıyla kapalı sistem haline gelen kavanoz 37 C derecedeki etüve konur. Embriyoların incelenmesi için vidalı kapağının her açılışında % 5 CO_2 verme işlemi tekrarlanır.

Bu iki sistem içersinde toplam 286 embriyonun gelişmeleri sabah ve akşam olmak üzere (10.30–17.30) günde iki defa incelenmiştir. Mikroskobik incelemelerden de anlaşılacağı üzere her iki ortam da embriyoların gelişmelerinde yeterlidir ancak kullanımlarında bazı ufak sakınca ve yararların olduğu görülmüştür.

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Summary: *During the culture of mouse embryos in medium at pH 7.2–7.4 it is necessary to maintain a constant atmosphere of 5 % CO_2 in air. This can be accomplished equally well either by culturing the embryos in a cabinet with continuous flow of gas or by briefly gassing and sealing the cultures in an anaerobic jar. A total of 286 embryos were observed in this study. Embryonic development was assessed by microscopic examination until Day 6 (144 h) post coitus. No significant differences in the development or in the proportion of mature blastocysts hatching was observed following culture of one-cell mouse embryos in this two systems. They were examined and scored for development at two times (10.30–17.30) every day during survey. However both systems have some inherent handicaps and advantages.*

Introduction

The increasing interest in the cultivation of fertilized mammalian embryos has urged scientists to determine the special nutritional requirements of the embryo during the early cleavage stages. During the last 50 years culture of the embryo has become the most popular model used in embryological research (2,3,4,6,8,12,13,15).

In 1949 Hammond (5) was the first worker to successfully culture 8 cell mouse embryos to blastocysts. As a medium he employed a salt solution containing sodium chloride, potassium chloride and magnesium chloride with a glucose concentration of 1 mg supplemented with about 5 % egg white as a macromolecular component. Hammond (5) put 1 to 6 embryos in small vessels in 2 to 3 ml of medium. Later this method was used and further developed by many research workers to successfully culture mouse and rabbit embryo smost of the pre-implantation period (1,2,3,7,10,12,14,16).

Since the mammalian embryo is highly adapted to the maternal environment the pH should be kept an optimal 7.4 ± 0.5 by equilibration with 5 ± 0.5 % CO_2 and 95 % air at 37° C. The CO_2 concentration in the incubator is therefore critical and this report examines the relative efficiency of two gassing systems on cleavage of the mouse embryo in a chemically defined culture medium (1,2,4,11,16).

Materials and Methods

One cell embryos were obtained from superovulated F_2 hybrids (8 weeks old) of C57Bl / 6J ♀ x CBA / Ca ♂ maintained under stan-

dard labratory conditions at the AFRC Institute of Animal Physiology, Animal Research Station, Cambridge. The yield of embryos was increased considerably by initial priming with gonadotrophins. An intraperitoneal injection of 5 I.U. Pregnant Mare's Serum Gonadotrophin (PMSG, Folligon, Intervet) was followed 48 hours later by 5 I.U. of Human Chorionic Gonadotrophin (HCG, Chorulon, Intervet). After HCG injection the females were paired with males. Mating was confirmed the following morning (= Day 1) by the presence of a vaginal plug.

During the morning of Day 1 mated females were autopsied and the oviducts were dissected and covered with a drop of hyaluronidase in a sterile petri dish. The oviducts were examined under a stereo dissecting microscope and the eggs were located in a cumulus clot in the ampullary portion.

The cumulus cell mass and eggs were released from the ampulla using a fine needle (25 gau.) and watchmaker's forceps. The eggs were released from the cumulus cells following incubation at 370° C for 2–3 minutes in the enzyme solution.

The embryos were removed from the enzyme solution and washed 6 times with M16 + BSA and finally cultured in M16 + BSA (Bovine Serum Albumin) under paraffin oil (6,8).

The embryos were then cultured in one of the following two systems maintained in a constant 37° C incubator:

1. This system consisted of a transparent perspex cabinet with removable shelves and a basal inlet and top outlet gas point (Fig.1). A continious flow of air generated by an aquatic aeration pump was mixed with CO_2 from a controllable cylinder and valve attachment to give a measured air flow rate of 5 % CO_2 in air. Prior to entering the culture cabinet the gas mixture was passed through a cylinder of water to increase the humidity and also serve as a final indicator of the flow rate.

2. A commercially available anaerobic jar (Baird and Tatlock, Ltd. London) with controllable inlet and outlet valves was used (Fig.2). The jar containing the cultures was gassed for 10 minutes from a cylinder containing 5 % CO_2 in air, sealed and placed in a 37° C incubator. When the cultures were removed for examination the jar was removed

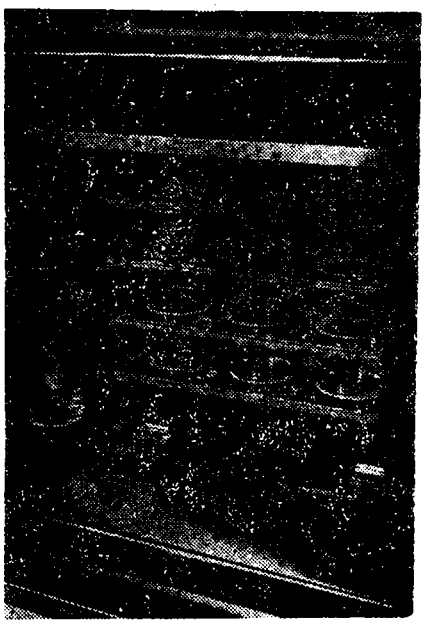

Fig.1. The transparent embriyo culture cabinet.
Sekil 1. Transparent embriyo kültür kabini.

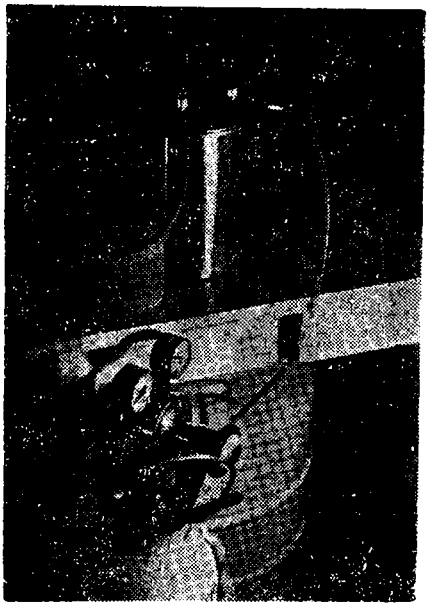

Fig. 2. The anaerobic jar.
Sekil 2. Anaerobik jar (Anaerobik kavanoz).

from the incubator and required regassing before returning to the incubator.

A total of 286 mouse embryos were observed in this study. The embryos were cultured up to stage of blastocyst hatching. They were examined and scored for development at two times (10.30 and 17.30 hours) every day during the survey.

Results

No significant differences in the development or in the proportion of mature blastocysts hatching was observed following culture of 1 cell mouse embryos in the two system tested.

Embryonic development was assessed by microscopic examination until Day 6 (144 hours) post coitus. During this time 257 (% 90) of the embryos had reached the blastocyst stage by the 4th day (96 hours) p.c. and 229 (89 %) hatched before the 5 th day (120 hours) p.c. Thus the entire process of preimplantation development in vitro was completed in 4–4 1 / 2 days (Fig. 3–4).

During use it was found more convenient to work with the incubator installed cabined which also had a larger capacity for individual culture dishes (36 petri dishes in the cabinet but only 12 in the anaerobic jar). The jar also cooled markedly during the observation periods when it was necessary to remove it from the incubator.

Although a continuous gas flow is required by the cabinet system the CO_2 cylinder used is much cheaper compared with the 5 % CO_2 in air cylinder used to gas the anaerobic jar cultures.

Discussion

The results demonstrated a consistently uniform rate of cleavage throughout and hatching of the majority of the blastocysts.

Most workers agree that the entire process from 1 cell to blastocyst is completed in 4 – 4 1 / 2 days (96 – 108 hours p.c.) and that was fully confirmed in the present study irrespective of the culture chambers used (6,8,9,17).

However both systems have some inherent handicaps and advantages.

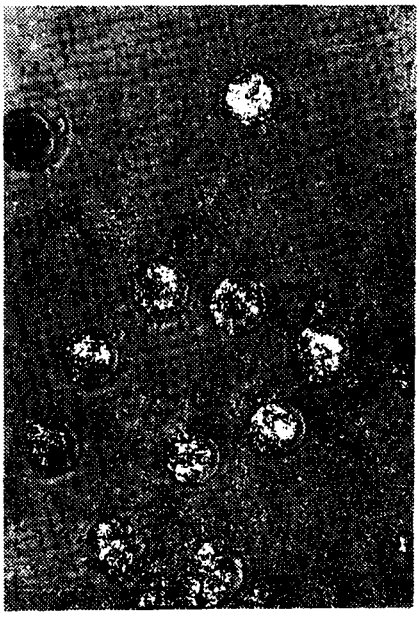

Fig. 3. One-cell mouse embryos in M16 culture medium. 180x
Sekil 3. M16 kültüründeki tek hücreli embriyolar.

Fig. 4. Hatching of the mature blastocysts. 360x.
Sekil 4. Açılım yapan olgun blastosüstler.

Although the anaerobic jar required relatively little gas compared with the continious flow system of the cabinet method, the gas for the jar was more expensive.

The mouse embryos developed well in both types of system, but the removal of the jar from the incubator and the need for a final 10 minutes gassing period following examination of the cultures means that an undesirable fall in temperature must have occured which may be critical on culturing more sensitive embryos from another species.

The cabinet system offered greater capacity for embryo culture, was much easier to use and is considered the system of choice.

References

1. **Biggers, J.D., Whitten, W.K., Whittingham, D.G.** (1971): *The culture of mouse ova in vitro.* In "Methods of Mammalian Embryology" Ed. J.C. Daniel Jr. Freeman San Francisco.

2. **Brinster, R.L.** (1963): *A method for in vitro cultivation of mouse ova from two cell to blastocyst.* Expl. Cell. Res. 32, 205.

3. **Brinster, R.L.** (1965): *Studies on the development of mouse embryos in vitro. IV Interaction of energy sources.* J. Reprod. Fert. 10, 227.

4. **Brinster, R.L.** (1969): *Mammolian Embryo Culture.* In "The Mammalian Oviduct" Ed. E.S.E.Hafez, R.J. Blandau. Univ. of Chicago Press. 419.

5. **Hammond, J.** (1949): *Recovery and culture of tubal mouse ova.* Nature Lond. 168, 28.

6. **Kılıçoğlu, S.Ç.** (1986): *The in vitro cultivation of mouse ova from one cell to blastocyst.* A.Ü. Vet. Fak. Derg. 32 (2) 301–310.

7. **McLaren, A., Biggers, J.D.** (1958): *Successuful development and birth of mice cultivated in vitro as early embryos.* Nature Lond. 182, 877.

8. **Rafferty, K.A.** (1970): *Methods in Experimental Embryology of the Mouse.* The Johns Hopkins Press. Baltimore - London.

9. **Streffer, C., Van Beuningen, D., Molls, M., Zamboglou, N., Schultz, S.** (1980): *Kinetics Sf cell proliferation in the preimplanted mouse embryo in vivo and in vitro.* Cell Tissue Kinet. 13, 135.

10. **Tarkowski, A.K.** (1961): *Mouse chimaeras developed from fused eggs.* Nature Lond. 190, 857.

11. **Wale s, R.G., Ouinn, P., Mkrdock, R.N.** (1969): *The fixation of carbon dioxide by the eight cell mouse embryos.* J. Reprod. Fert. 20, 541.

12. **Whitten, W.R.** (1956): *Culture Sf tubal mouse.* Nature Lond. 177, 96.

13. **Whitten, W.K.** (1971): *Nutrients requirements for the culture of pre-implantation embryos in vitro.* In: "Advances in the Biosciences." Vol. 6 Pergamon Press.

14. **Whitten, W.K., Biggers, J.D.** (1968): *Complete development in vitro of the pre-implantation stages of the mouse in a simple chemically defined medium.* J. Reprod. Fert. 17, 399.

15. **Whittingham, D.G.** (1970): *Biochemical aspects of early gestation* In: "Congenital Malformations". Proc. The Hauqe I.C.S. Excerpta Med. Fdn. 204, 119.

16. **Whittingham, D.G.** (1971): *Culture of mouse ova.* J. Reprod. Fert. Suppl. 14, 7.

17. **Zeilmaker, G.H.** (1981): *Embryo transfer in the mouse and in the rat.* In: "Frozen Storages of Laborotory Animals". Ed. G.H.Zeilmaker. Gustav Fisher Verlag. Stuttgart-New York 1981.