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A Simple Culture Device for the Culture and Transportation of Mouse Oocytes and Embryos in vitro(I)

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생쥐난자의 단기간 체외배양과 수송을 위한 Simple Culture Device

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적 요

포유류 동물 난자의 체외배양법이 발달되어 온 이래 趙 (1974)에 의한 미세관내 배양방법이 여러가지 장점을 가지고 있어 널리 이용되고 있다.

본 연구는 이 방법을 보다 간편하게 이용하는 방법과 이 방법을 이용하여 수송가능성을 제시하고저 행하였다.

본 연구의 결과, 사용한 simple culture device는 체외배양법으로서 매우 경제적인 한편 배양의 결과는 매우 양호하였다.

본 방법으로 배양중에 있는 난자의 수송이 가능함을 제시하였고, 난자의 성숙및 초기배아의 분화과정에 대한 연구를 손쉽게 여러 연구실에서 행할수 있을 것으로 기대된다.

INTRODUCTION

Several techniques for the culture of mouse oocyte and embryo were developed by many investigators since the pioneering work of Brachet (1912) who had cultivated rabbit ova *in vitro*.

Even though paraffin oil drop method (Brinster, 1963) has been widely adopted, there are many disadvantages in that method. In order to improve the method for studying the effect of oil-soluble substances on the oocyte maturation and embryogenesis, a microtube culture method (Cho, 1974) has some advantages; the isolation of medium from oil layer, simple and easy handling, and transportation.

There have been many efforts to develop the transporting or the storage

system for preserving viable cells, tissues, and organs by refrigeration, or by the low temperature freezing, or by the ultrarapid freezing (reviewed by Hafez, 1971; Andersen, 1974). But the devices for the storage and the transportation need quite special-equipments which are not available in the ordinary laboratories.

The present study is planned to improve the microtube culture method for easy transporting system while ova and embryos are still undergoing development and for short term culture within the laboratory which has less economical advantages.

MATERIALS AND METHODS

Ovaries were obtained from the immature A strain mice (3—4 weeks old kept under the controlled lightening conditions and temperature), randomly bred in our laboratory house for experimental animals.

For the culture, the oocytes were isolated from the Graafian follicles of ovary as described previously (Cho and Yoon, 1975). Main tool of this culture system was a 50 mm-long glass tube (internal diameter: 1 mm) obtained from the end of 9-inch-long disposable pasteur pipette (Harshow Chemicals) as shown in Figure 1A. Culture system was made by inserting 10 μl of modified Krebs-Ringer solution

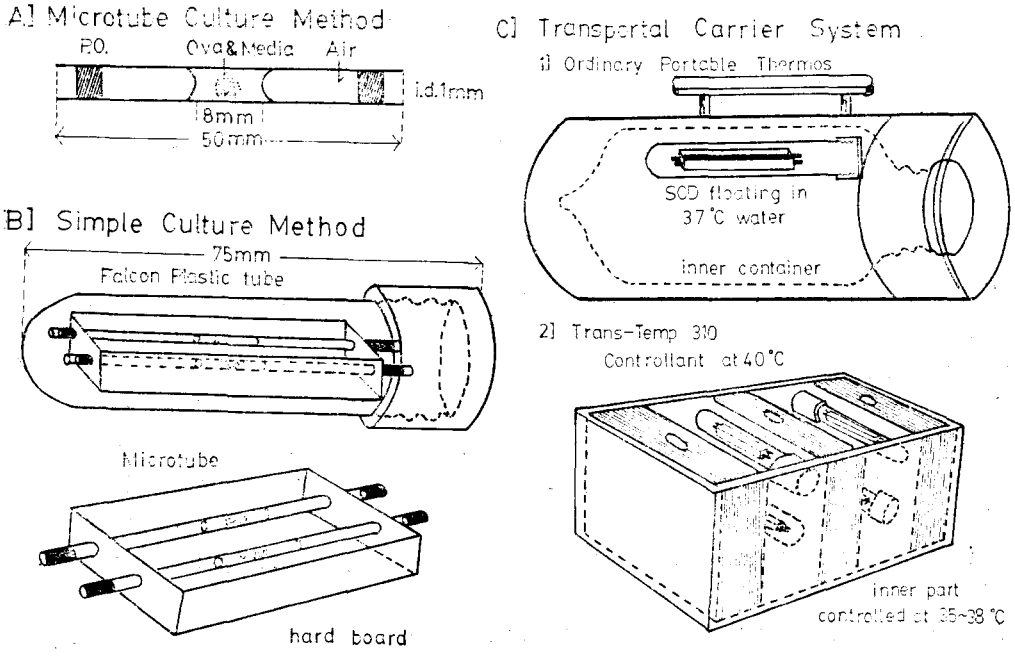

Fig. 1. Simple culture device and basis of microtube culture method

(Biggers *et al.*, 1971) sterilized with 0.45 μ Millipore filter into the middle part of microtube by a capillary pipette. The microtube was plugged at one end with 5 μl of paraffin oil, and incubated with 5% CO_2 at 37° C in fully moistened air condition for 2 hours to obtain the equilibration of the medium. After ten to fifteen ova and embryos were introduced into the medium under a stereomicroscope (Wild, M5A), the other open end was sealed immediately with paraffin oil, and then the tube was inserted into the holder in the test tube (Falcon plastic tube, 12×75 mm) as shown in Figure 1B. This simple culture device, of which cap was opened, was returned into the incubator for reequilibration.

After one or two hours incubation, the opened simple culture device was capped tightly and sealed with the vinyl tape, and then inserted into an ordinary thermos which was filled with prewarmed water at 37° C or into a Trans-Temp 310 (Royal Industry) which was designed for maintaining the contents at 35° C for three days (Figure 1C).

At the end of the culture period, the microtubes were picked up from the simple culture device and then the both ends of microtube were cut with a glass knife to remove the oil plugs. The middle piece of the tube was inserted into a micropipette adopter with rubber bulb (Drummone Scientific Co.) and the contents were carefully ejected into a drop of medium in a culture dish, and ova and embryos were examined under the stereomicroscope. Some of them were fixed with acetic alcohol and stained with 0.5% aceto-lacmoid solutions for further observation of nuclear progression of the ova or the development of the embryos.

RESULTS

Changes of inner temperature in these transporting carriers were checked at

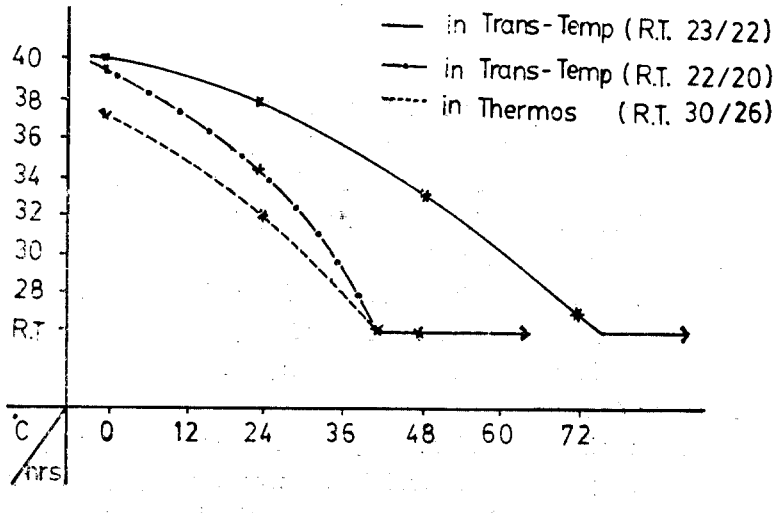

Fig. 2. Temperature range of the transportal carriers at the room temperature.

various room temperatures as shown in Figure 2.

The range of temperature in the Trans-Temp 310 was from 40° C to 28° C in summer season (room temperature, 26° C—30° C).

Ordinary thermos was more sensitive to the outer temperature. When ordinary thermos was filled with 37° C water, the temperature fell down to 30° C after 24 hours in summer and to room temperature (12° C) after 24 hours in winter.

The maturation rates of mouse oocytes in the simple culture device were compared with those in the ordinary microtube culture method.

The results were summarized in Table 1. The results showed that meiotic maturation was delayed in the simple culture device compared with the microtube culture method. But it is assumed clearly from these results that if the temperature is controlled constantly at 37° C, simple culture device system is acceptable for the short term culture of the mouse oocytes.

Table 1. Comparison of the maturation rates of the mouse oocytes cultured for 20 hours in the simple culture device (SCD) and in the microtube culture system (MCS).

System	Temperature	Total ova	Nuclear Phase		
			D/P	MI/TI	MII
SCD in thermos	37°C—30°C	64 (%)	13 (20.31)	31 (48.44)	20 (31.75)
SCD in incubator ($-CO_2$)	37°C	61 (%)	7 (11.48)	15 (24.59)	39 (63.93)
Microtube in incubator ($+CO_2$)	37°C	36 (%)	4 (11.11)	5 (13.89)	27 (75.00)

D/P: Oocytes in dictyate to prophase stage
MI/TI: Oocytes in metaphase I to telophase I stage
M II: Oocytes in metaphase II stage

In order to examine the possibility of transportation with this simple culture device, the maturation rates of oocytes in the ordinary incubator were compared.

Based upon the result summarized in Table 2, the maturation rates of oocytes in the simple culture device set-up in the Trans-Temp 310 system were similar to those in the incubator controlled at 37° C.

These results suggest that if the simple culture device is located in the controlled temperature at 37° C, the maturation rate of oocyte can be improved and oocytes and embryos can be mailed to the laboratory where is able to be received within 24 hours or 48 hours.

In order to test the availability of this system for embryo transportation, after two-cell mouse embryos were cultured in the transporting system or in the ordinary incubator and the rates of cleavage were examined. As shown in Table 3,

most of the two-cell embryos cultured in Trans-Temp 310 system were cleaved to four-cell during 24 hour incubation like those of control. The temperature of the Trans-Temp 310 system changed from 40° C to 34° C during this culture period in the laboratory of which room temperature could not be controlled.

Table 2. Comparison of the maturation rates of the mouse oocytes cultured for 24 hours in the simple culture device (SCD) within transportal system.

System	Temperature	Total ova	Nuclear Phase			Deg.
			D/P	MI/TI	MII	
SCD in incubator ($+CO_2$)	37°C	70 (%)	6 (8.57)	26 (37.14)	35 (50.00)	3 (4.39)
SCD in* Trans-Temp 310 system	40°C–36°C	257 (%)	34 (13.23)	96 (37.25)	117 (45.53)	10 (4.09)
SCD in** Trans-Temp 310 system	40°C–38°C	147 (%)	18 (12.24)	40 (27.21)	82 (55.78)	7 (4.77)

* : SCD was located on the bottom of the Trans-Temp 310 system
** : SCD was located on the bed of cotton in the Trans-Temp 310 system.
D/P: Oocytes in dictyate to prophase I stage
MI/TI: Oocytes in metaphase I to telophase I stage
M II: Oocytes in metaphase II stage
Deg.: Oocytes degenerated

Table 3. Cleavage of two-cell mouse embryos cultured for 24 hours in the simple culture device (SCD) within transportal system.

System	Temp. range	Developmental Stages			
		Total ova	2 Cell	3 Cell	4 Cell
SCD in incubator	37°C	47 (%)	1 (2.13)	2 (4.26)	44 (93.61)
SCD in Trans-Temp 310 System	40°C–34°C	67 (%)	5 (7.46)	—	62 (92.54)

But cleavage of the embryos were markedly deteriorated when the embryos were cultured in the system for 48 hours or 72 hours continuously. The temperature fell down from 40° C to 20° C during winter. This change may be the cause of cleavage delay or blocking in the carrier system (Table 4, 5). But these results suggest that this simple culture device could be used for the short term culture or for the transportation within 24—48 hours reach.

Table 4. Cleavage of two-cell mouse embryos cultured for 48 hours in the simple culture device (SCD) within transportal system.

System	Temp. range	Developmental Stages					
		Total	2—4C	8C	Mor.	Eb.	Abn.
SCD in incubator	37°C	37	14	—	20	3	—
		(%)	(37.84)		(54.05)	(8.11)	—
SCD in Trans-Temp 310 System	40°C—20°C	39	32	6	1	—	—
		(%)	(82.05)	(15.38)	(2.56)	—	—

2—4C: two to four cell embryos
8C: eight cell embryos
Mor.: morulae
Eb.: early blastocysts
Lb.: late blastocysts
Abn.: abnormal embryos

Table 5. Cleavage of two-cell mouse embryos cultured for 72 hours in various culture conditions.

System	Temp. range	Developmental Stages						
		Total(ova)	2—4C	8C	Mor.	Eb.	Lb.	Abn.
SCD in incubator	37°C	47	—	16	4	7	20	—
		(%)	—	(34.06)	(8.51)	(14.89)	(42.55)	—
SCD in* Transport System	40°C—20°C	67	10	11	26	12	—	8
		(%)	(14.93)	(16.42)	(38.81)	(17.91)	—	(11.94)
SCD in** Transport System and then in incubator	40°C—20°C	39	13	3	12	1	—	10
		(%)	(33.33)	(7.69)	(30.77)	(2.56)	—	(25.64)

*: Temperature of Trans-Temp 310 system was elevated to 40°C after 24 hours culture.
**: Embryos were cultured in the ordinary incubator for 24 hours after 48 hours culture in SCD within Trans-Temp 310 system.

DISCUSSION

Many techniques for the culture of ova and embryos were developed; paraffin oil drop method (Brinster, 1963), air compartment between oil and medium (Friedhandler, 1961; Mulnard, 1965), medium exposed to air (Whitten, 1971; Gwatkin and Haidri, 1973). But these are expensive culture methods for the ordinary laboratory experiments. Therefore, a less expensive culture method such as the microtube culture method is needed, which has many advantages compared with the most widely adopted method as shown in the following table.

Based upon the results of this study, it seems to be possible that the simple-culture device system is acceptable for short term culture of mouse oocytes,

Comparison of the culture method for the mouse oocytes

Criteria	Brinster's method (1963)	Cho's method (1974)
Culture type	plastic dish	microtube
Medium		
pH change	stable	very stable
evaporation	small	minimized
gas tension	good	excellent
Handling of ova	very simple	simple
Maintaining	expensive	very cheap
Transporting	impossible	possible
Study of oil soluble substances	impossible	possible
Observation during culture	very good	possible

even though the rate of the meiotic maturation was lower than that of microtube method.

It has been well known that many investigators have developed various techniques to preserve the viable cells and to store mammalian oocytes. Especially for the practical use in the oocyte transfer experiment and for the long distance transportation of embryos, it is desirable to maintain them *in vitro* without any harmful effects.

Since the methods of preserving and transporting the sheep oocytes in the genial tract were used (Adams *et al.*, 1961; Hunter *et al.*, 1962), refrigerating at the subnormal temperature and transporting the embryos have been investigated (Whittingham, 1971, 1972; Anderson, 1974) but these methods have had little success. Until now, the most successful results of the storage, transportation, and recovery have been obtained from the storaging embryos at or near −196° C by ultrarapid freezing (Whittingham, 1974, 1976; Maurer, 1976). But in these methods, the special equipments, which are not available at the ordinary laboratory, are required.

This study shows that it is relatively easy to transport the oocytes for some distance while they are undergoing development. By developing this system, mailing of the embryos and oocytes for some distance and short term culture without any specialized system seem to be possible. Based upon these results, this system is also expected to be helpful for the studies of the embryogenesis.

SUMMARY

This simple culture device is based on the microtube culture method (Cho, 1974). The microtube culture system was placed in a plastic tube (Falcon, 12×75 mm) and the transporting system used in the present study were Trans-Temp 310 (Royal Industries, 209 D 135-1).

The simple culture device in the Trans-Temp 310 seems to be an available method for short term culture and some distance transportation of oocytes and embryos.

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