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The effect of culture medium volume on in vitro development of mouse embryos

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Abstract

Background: In the field of mammalian embryo culture, the putative influence of autocrine/ paracrine factor(s), produce by the embryos itself, is under investigation. A smaller medium drop can prevent dilution of this factor(s).

Objective: The objective of this study was to examine the effect of culture medium volume on in vitro development of mouse 2-cell embryos.

Materials and Methods: The embryos were obtained from female NMRI mice. To evaluate the effect of medium volume, groups of 16-20 late 2-cell embryos were cultured in 2, 5, 10, 20, 50 and 100 µl of drops of Ham's F10 medium for 72 h.

Results: Development to blastocyst stage in 50 and 100 µl of drop were significantly higher than this in any other volume ($p<0.001$). Almost a similar pattern was also observed for hatched blastocyst formation. However, the total number of cells in blastocysts, developing in different volumes, were not significantly different.

Conclusion: These results indicate that the optimal volumes of Ham's F10 medium for mouse early embryo development are 50 to 100 µl. However, volumes as small as 2 µl can successfully support mouse 2-cell embryo development to blastocyst and hatching stages.

Key Words: *Mouse embryo, Culture medium, Incubation volume*

Introduction

In order to support in vitro development, different mammalian embryos have been empirically cultured in drops of medium with different volumes (e.g., 10-500 μl) covered with mineral oil. Recent studies showed that the volume of medium and embryo density (number per unit volume) are important factors that influence early embryo development (1-5). In IVF, different volumes of medium and numbers of embryos per a definite of incubation drop, have been described in literature with different results among authors, but only a few have studied these parameters as variation.

Some published data have showed that micro-cultures (small volume of medium), may lead to improved early embryo development in different species (3,6). This effect may be interpreted to suggest the secretion of autocrine or paracrine growth or survival factors by the embryos to support itself and other embryos in their development (3,6-8). A smaller incubation volume could prevent a dilution of these specific embryo-derived factors. However, some other studies could not support the idea of autocrine/paracrine stimulation of embryo development (4,9,10), and even have shown that culturing embryos in small volumes of culture medium is detrimental to their development (1,11). The objective of this study was to examine the effect of culture medium volume on in vitro development of mouse 2-cell embryos.

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Materials and Methods

Embryo collection

The embryos were obtained from 6 to 8-weeks female NMRI mice, which exhibit the two-cell block in vitro. The females were superovulated by an intraperitoneal injection of equine chorionic gonadotrophin (7 IU; eCG: Sigma, St. Louis, MO), followed by human chorionic gonadotrophin (7 IU; hCG: Profasi, Serono Laboratories, Inc.), 48hr later. They were then placed individually with males of the same strain. The following morning, the presence of a vaginal plug was confirmed. Late 2-cell embryos were obtained at 42-44 h after hCG administration by flushing the oviducts. HEPES buffered human tubal fluid medium (HTF; Irvine Scientific, Irvine, CA) medium was used for

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flushing and embryos collection. The embryos were rinsed 3 times in HTF and another 3 times in Ham's F10 (Gibco, Grand Island, NY), then were transferred into the culture treatments.

Experimental design

To evaluate the effect of medium volume on early embryo development, 2, 5, 10, 20, 50 and 100 μl drops of Ham's F10 with human serum albumin (5%) (HAS,Vitrolife, Sweden AB, Kungsbacka, Sweden) were placed in a single 60mm falcon dish (Falcon plastics, no. 1007) and covered with about 10ml mineral oil. A microsampler was used to prepare the drops of medium. All medium drops were equilibrated overnight at 37°C under 5% CO2 in air before culture began. All collected embryos from different animals were harvested in a 100 μl drop of Ham's F10 prior to distribution to treatment groups. Harvested embryos were randomly allocated into the culture drops in groups of 16-20 and cultured for 72 h at 37°C in 5% CO2 in air. When embryos were transferred into 2 and 5 μl drops, the culture medium volume in each drop was maintained by removing a volume equal to the volume of transfer medium added. Embryonic development was scored every 24 h and the proportions of the 4 to 8-cell, morula, blastocyst and hatching stages were recorded.

A total of 7 replicate were conducted (including all experimental groups) to standardize procedures and minimize experimental variations.

Determination of cell number

Embryos were placed into 0.9% (v/v) of sodium citrate (in water) for 10-20 min and fixed with a solution of ethanol: acetic acid (2:1) for 2 min. The fixed blastocysts were transferred to slides, air-dried and stained with 10% (w/v) Gimsa (Sigma) and the cell numbers were determined under a microscope.

Statistical analyses

The proportion of 2-cell embryos developing to the blastocyst stage, and the proportion of hatched blastocyst between groups were analyzed with the chi-square test.

Data on cell number of blastocysts from different groups were analyzed by ANOVA. Statistical analyses were conducted with the SPSS software package (SPSS 10).

Results

The proportions of the 2-cell embryos which developed to the blastocyst and hatching stages in different volumes of culture medium are shown in table I.

No differences were observed for the rate of blastocyst stage between 2, 5, 10 and 20 μl volumes of culture medium (68.8, 62.1, 62.2 and 67.2% respectively). However, significantly more embryos developed to blastocyst stage in 50 μl (81.5%) and 100 μl (79.0%) volumes as compared to any other volumes ($p<0.001$). A similar pattern was also observed for hatched blastocyst formation, except that there was no difference between 20 μl and 100 μl volumes and also there were significant differences between 20 μl volume and all smaller volumes ($p<0.002$). The mean (±SEM) number of blastomere per blastocyst were 35.9 ± 4.6, 41.3 ± 12.3, 37.6 ± 13.8, 38.1 ± 11.1, 42.6 ± 12.9 and 37.5 ± 8.0 in 2, 5, 10, 20, 50 and 100 volumes respectively. Which the differences were not significant.

Table I. Effect of the volume of Ham's F10 medium on mouse 2-cell embryo development after 72 h culture.

Volume (μl)	N*	Proportion (% ± SEM) of embryo developing to :	
		Blastocyst	Hatching
2	128	68.8 ± 5.6[a]	20.3 ± 6.2[a]
5	132	62.1 ± 5.4[a]	19.7 ± 2.1[a]
10	135	62.2 ± 8.4[a]	23.7 ± 16.5[a]
20	134	67.2 ± 7.3[a]	30.6 ± 11.3[b]
50	135	81.5 ± 4.2[b]	37.8 ± 16.3[c]
100	138	79.0 ± 1.0[b]	34.1 ± 11.6[cb]

* *Total number of 2-cell embryos cultured in 7 replicates; within each replicate 16-20 embryos were cultured in each volume.*

[abc] *Different superscripts differ significantly within the same column (first column: $p<0.001$, second column: $p<0.05$).*

Discussion

Some previous studies have shown that mammalian early embryo development and quality were improved by culturing the embryos in reduced volumes and/or by increasing embryo density (the embryo medium volume ratio) (3,6,12,13). To interpret this effect, the authors assumed that preimplantation embryos produce some mitogenic or embryotrophic factor(s) which enhances their development, specially the blastocyst and hatching rates (6-8,12,14). In

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smaller incubation volume the concentration of these specific embryo-secreted factor(s) increases sufficiently to exert its effect. However, other experiments have also shown that depending on the culture conditions, single embryos can be produced with the same developmental competence as embryos produced in groups (4,15,16) and also developmental rates were significantly higher in large volume (100 μl) than those in small volume of culture medium (1).

Our results does not support the idea of autocrine/paracrine stimulation of embryo development. Because two-cell embryos cultured in small volumes (2-20μl) of medium had significantly lower development than those cultured in 50μl and 100μl volumes. These results may be interpreted to suggest either an accumulation of toxic metabolites in the smaller volume medium (17) or depletion of medium components essential for development (18). The interaction between medium volume and culturing embryos can be divided into two aspects: positive conditioning, representing embryo-derived beneficial factors that stimulate their own development (Cooperative interaction), and negative conditioning, that produces detrimental effects on embryo development via accumulation of embryotoxic elements in the culture medium (11), or changing some critical environmental conditions such as PH and the osmolarity of medium drops. Variations in experimental conditions could account for some differences between our data and those published by other groups. It can be supposed that the reduced volume of incubation medium may show two adversed effect on embryo development in comparing to higher volume: In well quality controlled experimental conditions the positive cooperation of cultured embryos may acts more effectiveness in smaller droplet of medium; on the contrary, in low controlled conditions accumulation of the detrimental agents and changing the optimal conditions over to embryos tolerance, occurred sooner in small drops than larger one. Therefore, we suggest that the optimal incubation volume for embryo culture of the same species is different depending to culture conditions include type of medium and laboratory conditions.

The results of this study also showed that although large volumes of Ham's F10 medium has advantage for mouse embryo development, interestingly, the ultra-microdroplets (2-5µl) of medium could support early embryo development up to blastocyst and hatching stages. More importantly, the quality of the blastocysts were not different between the ultra-microdroplets and large drops because the total number of cells per blastocyst were not different between two groups. This data are on contrary to which reported by Kito *et al* (1997) (1). Their study showed that 2µl volume of culture drop only support the first cleavage and 5µl volume up to 3 cleavage of hamster zygotes. This controversy may be caused by interspecies differences and also difference in culture medium.

In conclusion, the results indicated that, medium volume influence the embryo development in mouse and the optimal volumes of Hum's F10 medium for blastocyst development of mouse 2-cell embryos are 50 to 100 μl. However, volumes as small as 2 μl could successfully support mouse 2-cell embryo development to blastocyst and hatching stages.

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