Optimization of Embryo Density and the Volume of Culture Medium for an Improvement of Mouse Parthenogenetic Embryo Development

Sangho Roh\textsuperscript{2,3,4}, Young-Joo Choi\textsuperscript{2,4} and Byung-Moo Min\textsuperscript{1,2,3,4,*}

\textsuperscript{1}Department of Oral Biochemistry

ABSTRACT

Autocrine or paracrine mediators released by the early embryo are implicated in the support of embryonic development. Their mechanisms and optimal embryo density in the medium, however, are uncertain. This study was conducted to establish the optimal embryo density and culture medium volume in mouse parthenogenetic embryo culture. In experiment 1, culture of parthenogenetically activated oocytes at a concentration of 2−4 embryos/\mu L significantly improved development to the blastocyst stage (72\% ≤) compared with culture at the lower (0.2−1 embryos/\mu L, 0−37.5\%) and the higher (5−6 embryos/\mu L, 30−53\%) concentration for 120 h when the oocytes were cultured in a 5 \mu L drop under mineral oil. In experiment 2, the embryos cultured at a concentration of 2−4 embryos/\mu L in a 10 \mu L drop (81.1\%) showed significantly higher blastocyst rates than those in a 5 \mu L drop (68.5\%). This study optimizes in vitro culture condition by modifying embryo density and the volume of culture medium. It may give appropriate level of autocrine and/or paracrine factors to enhance viability and subsequent normal development of mouse parthenogenetic embryos in vitro.

(Key words: Parthenogenesis, Embryo density, Culture medium volume, B6D2 F\textsubscript{1} mouse)

INTRODUCTION

The relative autonomy of preimplantation embryo growth, together with accumulating evidence for a role of released embryo-derived factors (Wiley et al., 1986) in supporting embryonic development, has provided support for a theory of regulation of embryonic development by autocrine/paracrine growth factors. Recent studies have implicated a variety of putative growth factors including platelet-activating factor (PAF; O'Neill, 1998; O'Neill, 1997), insulin-like growth factor I (IGF-I; Schultz et al., 1993; Harvey and Kaye, 1992a), IGF-II (Harvey and Kaye, 1992b), and epidermal growth factor/transforming growth factor-\alpha (Woods and Kaye, 1989; Babalola and Schultz, 1995). Their mechanisms of action is poorly understood, while the very nature of autocrine/paracrine mediators (produced by and acting on the same cell populations) make such studies difficult. It was proposed that combined use of in vitro fertilization and culture at low embryo concentration provides a functional, multiple-growth factor ablation model for defining the role of autocrine/paracrine factors in early embryonic development (O'Neil, 1997). Using this model, O'Neil (1998) showed that exposure of embryos to autocrine/paracrine factors during the 2-cell stage is necessary for subsequent normal development to the blastocyst stage. Deprivation during this time led to subsequent retarded development with an increased incidence of cell death in embryos. However, the embryos cultured at higher embryo concentration may produce more metabolites or oxygen radicals than those at lower concentration, which is detrimental against further embryonic development. Mechanisms of autocrine/paracrine factors and metabolites in the medium, however, are still uncertain. Hence, optimization of embryo concentration in the medium is required to increase efficacy of viable embryo

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\textsuperscript{2} Craniofacial Reconstructive Science Major.
\textsuperscript{3} BK21 HLS.
\textsuperscript{4} Dental Research Institute, Seoul National University College of Dentistry, Seoul 110-749, Korea.
\textsuperscript{*} Corresponding author : Dr. B. M. Min, Department of Oral Biochemistry and Craniofacial Reconstructive Science, Seoul National University College of Dentistry, 28 Yeonkun-Dong, Jongno-gu, Seoul 110-749, Korea. TEL: +82-2-740-8661, FAX: +82-2-740-8665, E-mail: bmmin@snu.ac.kr
production in vitro.
This study was conducted to establish the optimal embryo density in F1 hybrid (C57BL/6 × DBA/2; B6D2 F1) mouse parthenogenetic embryos. In addition, we investigated whether different volume of medium with same embryo concentration affected mouse parthenogenetic embryo development.

**MATERIALS AND METHODS**

**Reagents and Media**
All inorganic and organic compounds were purchased from Sigma-Aldrich (St Louis, MO, USA) unless otherwise stated. All the media used here were based on CZB and KSOM media (Nagy et al., 2003).

**Recovery of Oocytes**
Eight-week old B6D2 F1 mice were superovulated by intraperitoneal injections of 7.5 IU equine chorionic gonadotropin (eCG) and 7.5 IU human chorionic gonadotropin (hCG) given 48 h apart. Superovulated females were killed by cervical dislocation at 16 h after hCG injection, and the oviducts were removed and transferred into a Petri dish containing 2 mL Hapes-buffered CZB medium (HCZB) supplemented with 300 IU/mL hyaluronidase (bovine testis). The oviduct ampullae were opened, and the cumulus-enclosed oocytes were released. After 2–3 min exposure to the medium, the cumulus-free oocytes were washed twice in HCZB before activation.

**Activation and In Vitro Culture**
Recovered oocytes were then immediately exposed to activation medium consisting of 10 mM SrCl2 with 5 μg/mL cytochalasin B in calcium-free CZB for 5 h or cultured in CZB in an atmosphere of 5% CO2 in air. Following this activation the oocytes were cultured in KSOM at 37°C under mineral oil in an atmosphere of 5% CO2 in air. Detailed experimental design is described below. Activated and in vitro cultured oocytes were then recorded the rates of their development to the blastocyst stage after 120 h of culture.

**Statistic Analysis**
Differences in the mean percentages of embryonic development among the treatments were analyzed by Chi-Square test.

**Experimental Designs**
In experiment 1, the activated oocytes were cultured in the medium of a 5 μL drop at concentrations of 0.2, 1, 2, 3, 4, 5 and 6 embryos/μL. In experiment 2, the oocytes were cultured at a concentration of 2–4 embryos/μL either in a 5 or 10 μL drop.

**Animal Ethics**
All animal experiments were approved and performed under the guidelines of Institutional Animal Care and Use Committee in Seoul National University.

**RESULTS**
In experiment 1, culture of parthenogenetically activated oocytes at a concentration of 2–4 embryos/μL significantly improved development to the blastocyst stage (72.0% ±) compared with culture at the lower (0.2–1 embryos/μL, 0–37.5%) and the higher (5–6 embryos/μL, 30.0–53.0%) concentration for 120 h when the oocytes were cultured in a 5 μL drop under mineral oil (p<0.05, Table 1). In experiment 2, the embryos cultured at a concentration of 2–4 embryos/μL in a 10 μL drop (81.1%, 73/90) showed significantly higher blastocyst rates than those in a 5 μL drop (68.5%, 202/295; p = 0.036, Table 2).

**DISCUSSION**
The present study aimed for an improvement of parthenogenetic embryo development in B6/D2 mice which is a prerequisite step in SCNT programme. The strain we chose (B6/D2) is known as one of the best strain for micromanipulation since their MI chromosomes are visible without the use of fluorescence dye and they are also tolerance to nuclear injection. To optimize in vitro

| Table 1. Effect of embryo density on in vitro development of mouse parthenotes |
|---------------------------------|-------|-----------------|
| Embryo density<sup>a</sup> (embryos/μL) | Total oocytes | Development to blastocysts by 120 h (%) |
| 0.2 | 6 | 0 (0.0) |
| 1 | 40 | 15 (37.5)<sup>b</sup> |
| 2 | 50 | 36 (72.0)<sup>b</sup> |
| 3 | 60 | 46 (76.7)<sup>b</sup> |
| 4 | 140 | 105 (75.0)<sup>b</sup> |
| 5 | 100 | 53 (53.0)<sup>a</sup> |
| 6 | 90 | 27 (30.0)<sup>a</sup> |

<sup>a</sup> Embryos were cultured in 5 μL drops under mineral oil.

<sup>b</sup> p<0.05.
Table 2. Effect of the size of medium volume on in vitro development of mouse parthenogenotes

<table>
<thead>
<tr>
<th>Volume of culture medium</th>
<th>Total oocytes</th>
<th>Development to blastocysts by 120 h (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 μL</td>
<td>295</td>
<td>202 (68.5)(^a)</td>
</tr>
<tr>
<td>10 μL</td>
<td>90</td>
<td>73 (81.1)(^b)</td>
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\(^a\) p=0.036.

in vitro. Further experiments will define the mechanisms of action of autocrine/paracrine factors required for normal development of mouse parthenogenetic embryos.

REFERENCES


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