—Research Note—

***In Vitro* Development of Mouse Parthenogenetic Embryos to Blastocysts: Effect of Embryo Density**

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Abstract. Mouse haploid and diploid parthenogenetic embryos were cultured in 25 μ l of Whitten's medium using 1, 5, 10 or 20 embryos. Improvement in development was observed at higher embryo densities. Development of haploid parthenogenetic embryos was improved in terms of both the percentage of blastocysts and the number of cells per blastocyst. In diploid parthenogenetic embryos, an increase in the number of cells per blastocyst was observed. These results demonstrate that the development of parthenogenetic embryos is affected by embryo density. *In vitro* culture of 10–20 parthenogenetic embryos together can enhance their preimplantation development.

Key words: Development, Embryo density, Parthenogenetic embryo, Mouse.

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Parthenogenetic embryos have been used in animal reproduction through the multiplication of embryos aggregated with a single blastomere from a normal embryo [1, 2]. Thus, improving development of parthenogenetic embryos may be advantageous for this embryo multiplication technique.

Recently, several reports have demonstrated that the development of fertilized embryos can be enhanced by increasing the number of embryos in the culture drop for mice [3–5] and rabbits [6]. Thus, the development of parthenogenetic embryos may be improved by this effect. This study was undertaken to investigate this possibility.

Materials and Methods

Collection of oocytes

F1 (C57BL/6 $\times$ CBA) female mice were super-

ovulated by injection of 5 IU PMSG (Serotropin, Teikoku Zoki, Japan) followed 48 h later with 5 IU hCG (Gonotropin, Teikoku Zoki, Japan). The unfertilized oocytes were collected 16.5–17.5 h after hCG injection by piercing the swollen ampulla of oviducts in Whitten's medium [7] supplemented with 0.11 mM Na₂EDTA [8] (WM+EDTA) under paraffin oil.

Parthenogenetic activation and culture

The cumulus cells were removed by 150 IU/ml hyaluronidase (Type I-S, Sigma, USA) and washed twice. The cumulus-free oocytes were exposed to 7% ethanol (Kanto Chemical Co., Inc., Japan) [9, 10] in WM+EDTA for 1 min, then washed 8–10 times with WM+EDTA. The oocytes were further incubated in WM+EDTA (to produce haploids) or WM+EDTA with 0.5 μ g/ml cytochalasin-B (Sigma, USA) (to produce diploids) for 6–8 h in 5% CO₂ in air at 37 C. After incubation, haploid and diploid parthenogenetic embryos of normal morphology were selected and placed in drops of 25 μ l

WM+EDTA, varying the number of embryos (1, 5, 10 or 20 embryos/drop). The parthenogenetic embryos were further cultured in 5% CO₂ in air at 37 C for 96 h (diploid) or 120 h (haploid). The embryos that developed to the blastocyst stage were fixed for cell counting by the method described previously [11].

Statistical analysis

Comparison between groups was made using Student's *t*-test.

Results

Both the percentage of blastocysts and the number of cells per blastocyst in haploid parthenogenetic embryos increased at higher embryo densities (Table 1). Cultures with 10 and 20 embryos produced significantly larger numbers of blastocysts ($p<0.05$) than those with 1 and 5 embryos per drop. The cell number increased gradually as the embryo density increased but no significant difference between 5 and 10, or 10 and 20 embryos per drop was observed.

In diploid parthenogenetic embryos, no significant difference in the percentage of blastocysts was observed (Table 2). However, the cell number increased gradually as did that of haploid parthenogenetic embryos.

Discussion

This study clearly demonstrates that embryo density affects the *in vitro* development of mouse parthenogenetic embryos. Culture of 10–20 embryos together can enhance their preimplantation development.

It was suggested that cooperative interactions via some self-releasing growth factor(s) occur among embryos [4, 5]. After parthenogenetic activation, the parthenogenetic embryos showed protein synthesis similar to that of fertilized embryos [12, 13]. Therefore, the present results suggest that the parthenogenetic embryos may synthesize certain growth factors, since some growth factors are detected as maternal transcript products in the unfertilized ovulated oocytes [14, 15].

It is also possible that an increase in local CO₂ might account in part for the improvement at higher embryo density as suggested by Bavister [16]. The improvement in the rate of blastocyst development also occurs under increased CO₂ concentrations during culture *in vitro* in the hamster [17] and rabbit [18], but not in the mouse [19]. In mice, the O₂ concentration has been shown to affect *in vitro* development, and lower concentrations give better results [7, 20]. This may also be involved in the developmental improvement caused by increasing the embryo density.

Table 1. Effect of embryo density on the development of haploid parthenogenetic embryos

No. embryos/ drop (25 μ l)	No. replicates (no. embryos)	% Blastocysts (mean $\pm$ SEM)	Cell number (mean $\pm$ SEM) (no. embryos)
1	4(113)	28.9 $\pm$ 3.0 ^a	65.5 $\pm$ 2.6 ^d (31)
5	4(125)	46.5 $\pm$ 3.9 ^b	77.5 $\pm$ 3.5 ^e (55)
10	4(110)	68.3 $\pm$ 3.5 ^c	86.7 $\pm$ 3.7 ^{e,f} (71)
20	4(140)	62.6 $\pm$ 5.9 ^c	91.7 $\pm$ 3.7 ^f (79)

Values with different superscripts are significantly different: a-b-c, $p<0.05$; d-e-f, $p<0.01$.

Table 2. Effect of embryo density on the development of diploid parthenogenetic embryos

No. embryos/ drop (25 μ l)	No. replicates (no. embryos)	% Blastocysts (mean $\pm$ SEM)	Cell number (mean $\pm$ SEM) (no. embryos)
1	4(69)	87.6 $\pm$ 5.6	54.5 $\pm$ 1.6 ^a (59)
5	4(65)	93.7 $\pm$ 0.4	60.6 $\pm$ 1.8 ^b (61)
10	4(60)	96.2 $\pm$ 2.4	63.7 $\pm$ 2.1 ^{b,c} (56)
20	4(80)	92.5 $\pm$ 2.5	68.0 $\pm$ 1.7 ^c (74)

Values with different superscripts are significantly different: $p<0.05$.

The developmental potential of haploid parthenogenetic embryos is inferior to that of diploid parthenogenetic embryos in terms of both cleavage rate and blastocyst development [21]. The presence of only one genetic composition and an altered nuclear/cytoplasmic ratio may lead to a slower cleavage rate and a smaller number of blastocysts [21–23]. In addition, the environmental conditions such as growth factors and/or gas atmosphere may be very important for the development of haploid parthenogenetic embryos.

The results of this study suggest that embryo

density should be considered for culturing manipulated embryos using parthenogenetic embryos to assist development.

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