Embryo Density and Medium Volume Effects on Early Murine Embryo Development

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Submitted: May 22, 1992
Accepted: August 21, 1992

Background: One-cell mouse embryos were used to determine the effects of drop size and number of embryos per drop for optimum development in vitro.

Methods: Embryos were collected from immature C57BL6 female mice superovulated with pregnant mare serum gonadotropin and human chorionic gonadotropin and mated by CD1 males. Groups of 1, 5, 10, or 20 embryos were cultured in 5-, 10-, 20-, or 40-μl drops of CZB under silicon oil at 37.5°C in a humidified atmosphere of 5% CO2 and 95% air.

Results: Development score for embryos cultured in 10 μl was higher than that of embryos cultured in 20 or 40 μl. Embryos cultured in groups of 5, 10, or 20 had higher development scores than embryos cultured singly. The highest development score was obtained by the combination of 5 embryos per 10-μl drop. The percentage of live embryos in 20 or 40 μl was lower than that of embryos cultured in 10 μl. Additionally, the percentage of live embryos cultured singly was lower than that of embryos cultured in groups.

Conclusions: Our results suggest that a stimulatory interaction occurs among embryos possibly exerted through the secretion of growth factors. This effect can be diluted if the embryos are cultured in large drops or singly.

KEY WORDS: embryo culture; density; culture volume.

INTRODUCTION

The development of murine embryos in vitro is affected by factors such as strain of mice, culture medium, and culture system. Currently, most culture systems involve the use of microdrops of medium covered with oil. Brinster (1) demonstrated that it was possible to culture mouse embryos from the two-cell to the blastocyst stage in vitro using microdrops of medium covered with oil. Embryo density can affect murine preimplantation embryo development in vitro (2). Recently Paria and Dey (3) showed that the ratio of the number of embryos per microliter of medium affected preimplantation embryo development. Therefore, our objective was to establish the optimum volume of medium and number of embryos per drop for in vitro development of one-cell mouse embryos to the hatching or hatched blastocyst stage.

MATERIALS AND METHODS

Female C57BL6 mice (Charles River, Wilmington, MA), 3 to 4 weeks old, were superovulated with 5 IU pregnant mare serum gonadotropin (PMSG; Diosynth Inc., Chicago, IL) and, 48 hr later, 5 IU human chorionic gonadotropin (hCG; Schein Pharmaceuticals, Inc., Port Washington, NY) and mated by CD1 males. One-cell embryos (n = 640) were collected 20 to 22 hr after hCG. Pooled embryos (n = 40) were randomly assigned to 1 of 16 culture systems. Groups of 1 (n = 40), 5 (n = 8), 10 (n = 4), or 20 (n = 2) embryos were cultured in 5-, 10-, 20-, or 40-μl drops of CZB medium (4) under silicon oil at 37.5°C in a humidified atmosphere of 5% CO2 in air for 120 hr. Embryos were evaluated every 24 hr and scored 0 to 9 (0 = degenerate; 1 = two-cell; 2 = four-cell; 3 = eight-cell; 4 = compact morula; 5 = early blastocyst; 6 = blastocyst; 7 = expanded blastocyst; 8 = hatching blastocyst; 9 = hatched blastocyst). Score rather than cell number was evaluated to reduce the skewness of the treatments. Differences in development scores and percentage of live embryos (PLE) at each time period were analyzed by analysis of variance (5). Embryos were classified live if they were at the appropriate stage of in vitro development at each time of evaluation. Embryos that had degenerated or that had arrested were not considered advancing in development.

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RESULTS

The effect of medium volume on development score is shown in Table I. During the first 24 hr of culture, embryos cultured in the larger volumes developed more rapidly. By 72 and 96 hr embryo development was similar across culture volumes. However, the development score at 120 hr for embryos cultured in 10-μl drops (6.1 ± 0.3) was higher (P < 0.01) than that for embryos cultured in 20 μl (4.9 ± 0.3) or 40 μl (4.5 ± 0.3). The development score for embryos cultured in 5-μl drops (5.6 ± 0.3) was not different from that for embryos cultured in 10 or 20 μl but was higher (P < 0.01) than that for embryos cultured in 40-μl drops.

Embryo numbers began to have a significant impact on development by 96 hr in culture (Table II). Culturing embryos in groups of 5, 10, or 20 resulted in higher developmental scores (P < 0.01) than that of embryos cultured singly at 120 hr (5.5 ± 0.3, 5.6 ± 0.3, and 6.2 ± 0.3 vs 3.8 ± 0.3). The highest developmental score at 120 hr was obtained by the combination of 5 embryos per 10-μl drop (7.1 ± 0.6). The lowest development score was obtained by the combination of 1 embryo per 40-μl drop (3.0 ± 0.6; Fig. 1). Generally, multiple embryos in 10-μl had the highest development scores, while single embryos developed less except for single embryos in 5-μl drops. Variance between replicates generally decreased from 3.5² to 0.8² as the observation within a replicate increased from 1 to 20 embryos. However, when the data were pooled and evaluated by the ratio of embryos per unit volume, the ratio of 2 embryos/μl of medium resulted in the highest development score (Fig. 2). While not statistically different (P > 0.10), the ratios of 1 embryo/μl and 4 embryos/μl did not develop as well. The 2:1 ratio was greater (P < 0.05) than all ratios below 1 embryo/μl (1:1).

Beginning after 48 hr an 8 to 18% decline in PLE occurred at 72 hr (Table III). Percentage of live embryos after 120 hr of culture also was affected by (P < 0.05) both drop size and number of embryos per drop (Tables III and IV). At 120 hr the PLE cultured in 40 μl (58.7) was lower (P < 0.05) than that of embryos cultured in 10 μl (77.5). The PLE cultured in 5 μl (73.8) and 20 μl (64.4) was not different (P > 0.05) from the others. Additionally, the PLE for embryos cultured singly (51.3) was lower (P < 0.05) than that for embryos cultured in groups of 5 (72.5), 10 (70.6), or 20 (71.3) per microdrop.

DISCUSSION

This study confirms and expands upon previous reports on the effect of embryonic density on murine embryo development in vitro. Quinn et al. (6) cultured 1, 10, or 20 mouse zygotes in 10-μl drops and found that embryos cultured in groups had higher developmental rates to blastocyst and hatching blastocyst stages than embryos cultured singly. In our study, the combination of a 10-μl drop and 10, 20, or 40 embryos per drop yielded the best development.

Our results also support the theory that mouse embryos secrete compounds that are required for optimum development in vitro. Rapolee et al. (7) investigated the production of growth factors by mouse preimplantation embryos. They were able to detect mRNA for platelet-derived growth factor (PDGF) and transforming growth factor alpha (TGFα) and TGFβ1 in whole blastocysts, suggesting a role for these factors in early differentiation and development of mouse embryos.

More recently, Paria and Dey (3) showed that two-cell mouse embryos cultured singly in 25-μl drops had inferior development to the blastocyst stage and lower cell numbers per blastocyst than those cultured in groups of 5 or 10. Moreover, they were able to improve the inferior development of singly cultured embryos by adding epidermal...