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P-12 Hatching of Mouse Blastocysts on Somatic Cell Culture

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INTRODUCTION

Mammalian fertilized egg cleaves, develops to blastocyst, and escapes from zona pellucida for implantation and furthur development in uterus. Mouse embryos suffer from developmental block because the embryotoxic molecules such as oxygen radical generated under conventional culture condition.

A variety of culture conditions have been found to attenuate blocking phenomenon in vitro (Bongso et al., 1988) and to increase development of fertilized egg to blastocyst in the mouse embryos (Sakkas et al., 1989). Embryotrophic factor(s) produced by supporting cell layer was believed to promote the development of embryo in vitro. In those of experiments reporting the potentiation of embryonic development in somatic cell coculture, hatching of embryo also was increased. However, it was unclear that whether the increase in hatching resulted from potentiation of embryonic development or from hatching promoting factor(s) released by embryo proper or somatic cell culture.

To verify the precise role of somatic cell coculture on the embryonic hatching in vitro, two different types culture were examined thier effect on the hatching of mouse blastocyst; granulosa cell primary culture and Sertoli cell line. In earlier experiments, granulosa cell primary culture was used for embryo coculture and it was proven to enhance embryonic development in vitro. So far the Sertoli cell which plays important roles in nurishing and regulating the various aspect of spermatogenesis (Russel et al., 1993) has not been examined for their ability to support the embryonic development in vitro. TM_4 cell line originated form immature mouse testis has been known to have similar properties to Sertoli cell and secretes several growth factors with mitogenic effect (Holmes et al., 1986), metal ion binding protein such as transferrin (Skinner et al., 1980), and several proteinases (Bardin et al., 1993). Many of these molecules were known to potentiate embryo development *in vitro*.

Present study aims to monior the hatching of blastocyst on the somatic cell coculture and verify the effectiveness of Sertoli cell line coculture on the hatching of mouse blastocysts in vitro.

MATERIALS & METHODS

All chemicals were of the highest purity available commercially and were purchased from Sigma. Hormones was obtained from Sigma. FBS and DMEM was obtained from Gibco. TM_4 cell line was obtained form ATCC. and subculture was made for embryo coculture. TM_4 cell line was grown in DMEM (Gibco BRL) supplemented with 10 % FBS. Monolayer of TM4 cell line was obtained after 48 hr and used for embryo coculture. Granulosa cell was collected from immature mouse ovaries by puncturing the follicles in RPMI (10 % FBS) and confluent layers of primary culture was obtained 48 hr after seeding.

ICR mice were superovulated with PMSG (5IU) and hCG (5IU), and blastocysts were collected from uterus with retrograde flushing with M2 medium supplemented with 0.4% BSA on 96 hr after hCG injection. Embryos were placed in culture dish with or without somatic cell and incubated at 37C, 5% CO_2 in air in 100% humidity for 48 hr. Embryonic hatching was recorded 6 hr interval for 48 hr. Hatching was graded as 0 (no hatching), 1 (extrusion of samll bubble of blastomeres out of ZP), 2 (escape of half embryo from ZP), and 3 (complete hatching, escape of embryo from ZP).

RESULTS & DISCUSSION

Hatching rate was higher in the cocultured embryos and degeneration of embryos was greatly reduced by somatic cell coculture. It suggested that increment in embryonic hatching by somatic cell coculture was not cell type specific and involvement of common molecules in the potentiation of hatching of embryo on the two different somatic cells coculture. It has been

known that one of the detrimental factors accompanying the in vitro culture of preimplantion embryo was a production of oxygen radicals under high O_2 concentration. In this regard, somatic cells have been known to be a rich source of oxyradical scavenging molecules such as transferrin, superoxide dismutase, and catalase.

Interestingly, blastocysts cultured without the somatic cells started to hatch more faster than cocultured embryos during the first 12 hr of culture regardless of cell types cocultured. It was reported that the concentration of intracellular superoxide ion of mouse blastocyst suddenly increased prehatching period and minute quantity of exogenous hydrogen peroxide ehhanced the hatching (Thomas et al., 1997). We speculate that production and intracellular rise in oxygen radical concentration was virtually decreased in the blastocyst under somatic cells coculture, and it might have delayed the initiation of hatching of these embryo. However after 24 hr coculture onwards, progression of hatching in cocultured embryos was faster than control, and at the end of culture overall hatching rate of cocultured embryos was significantly higher than that of control. Now we expect the possiblity that modification of zona pellucida by proteinase or other biologically active molecules originated from somatic cell culture. Further study on the effect of somatic cell coculture on the structural and biochemical changes in zona pellucida will be helpful for comprehensive understanding the hatching promoting effect of somatic cell coculture. TM_4 cell line employeed in this experiment was effective for potentiation of hatching and prevention of degeneration of mouse embryo *in vitro*.

SUMMARY

Effects of somatic cell coculture on hatching of mouse blastocyst was examined. Mid-blastocysts were cocultured with granulosa cell primary culture or Sertoli cell line (TM_4) derived from mouse testis for 48 hr. Blastocysts cultured in medium (10% FBS) started to hatch more faster than cocultured embryos during 12 hr of coculture. After then blastocysts cocultured with somatic cell hatched faster than control. Degeneration of embryos was also greately reduced by coculture. This result suggested the potentiation of hatching as well as embryonic viability by coculture with somatic cell and Sertoli cell line can be used for embryo coculture.

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