Regulation of mouse embryo development by autocrine throphic factors

M.T. TERUEL1,2, R.C. CATALANO2, J.A. CABODEVILA2 AND S.S. CALLEJAS2

1Area de Embriología. 2Area de Reproducción, FISFARVET. Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, (B7000GHG) Tandil, Argentina.

Key words: embryo development, mouse, autoregulation, trophic factors.

ABSTRACT: Embryo development depends on maternal and embryonic factors. When occurs in vitro, embryos secrete factors that stimulate their development. The purpose of this study was to investigate the possible effects of embryos at morula stage on mouse embryo development in vitro. To obtain conditioned media (CM), morulas were cultured in groups of 5 (CM5) or 10 (CM10) in microdrops of Ham-F10 culture medium during 24 h and later they were removed. Subsequently, 365 morulas were cultured in CM5 and CM10 or in Ham-F10 media (as control group). No differences in blastocyst formation could be found between embryos cultured for 24h in Ham-F10, CM5 or CM10 (49.66, 53.04, 60.00% respectively). However, CM5 significantly increased differentiation in embryos cultured for 48h as compared to Ham-F10 medium (80.00% and 64.14 respectively). The CM5 caused a significant increase in the hatching rate compared to Ham-F10 evaluated at 78 and 96 h of culture (66.96 vs. 52.41% and 70.43 vs. 55.17%, respectively). After 72, 78 and 96h of culture the hatching rate for embryos cultured in CM10 was significantly higher than that in Ham-F10 (64.76 vs. 47.59%, 67.62 vs. 52.41% and 73.33 vs. 55.17%, respectively). At 48h of culture, differences between CM5, CM10 and Ham-F10 were not observed. These results suggest that preimplantational mouse embryos produce trophic factor/factors that enhance the differentiation and hatching process.

Introduction

Preimplantation mouse embryos are suitable for growing and differentiating in vitro in the absence of exogenous factors suggesting a role for endogenous factors that stimulate the development of them. Furthermore the embryos developing in vitro to the blastocyst stage, require more time and show a loss in the number of cells compared to embryos developing in vivo (Bowman and Mc Laren, 1970; Teruel and Smith, 1997) as response to suboptimal culture conditions. Embryo participation during early development has been proposed. Paria and Dey (1990) suggest that the retarded development of preimplantation embryos observed in vitro is a consequence of either the absence of growth factors of reproductive tract origin or the dilution of these factors released by the embryos in the culture medium or both. This indicates a function of autoregulation and possible cooperative interactions among embryos. According to these observations other investigators (Wiley et al., 1986; Lane and Gardner, 1992; Teruel and Smith, 1997; O’Neill, 1997; Rijnders and Jansen, 1999) have shown a beneficial effect on in vitro development of mammalian embryos cultured in groups, supporting the hypothesis that the embryo releases autocrine growth factors influencing the normal rates of embryo survival. Furthermore, the findings that preimplantational mouse embryos synthesize growth factors ligands and their

Address correspondence to: Prof. Miriam Teruel. Área de Embriología, Departamento de Ciencias Biológicas, Facultad de Ciencias Veterinarias, UNCPBA, Paraje Arroyo Seco s/n B7000GHG Tandil, Provincia de Buenos Aires, ARGENTINA. Tel./Fax: (+54-2293) 428667. E-mail: mteruel@vet.unicen.edu.ar Received on October 8, 2004. Accepted on March 16, 2005.
receptors (reviewed by: Teruel et al., 2000; Diaz Cueto et al., 2000; Hardy and Spanos, 2002), and that exogenous growth factors enhance in vitro embryo development (Paria and Dey, 1990; Teruel and Smith, 1997; O’Neill, 1997; Diaz-Cueto et al., 2000; Pantaleon et al., 2003), support that embryos could respond to different exogenous and/or endogenous growth factors.

There is increasing evidence that there exists a regulation by the embryos themselves during early development. However, the culture condition is sub optimal compared with maternal environment, that is the reason why the research for improving the culture media is constant. So, it gives rise to the importance of considering the influence of autocrine trophic factors as regulators of early embryo development.

The purpose of this study was to investigate the possible effects of conditioned media for embryos at morula stage on mouse preimplantation development in vitro.

Material and Methods

Animals

Female and males mice Balb C were maintained in a lighting regimen of 12 h light and 12 h dark during seven days before use. Female mice Balb C, (6-8 weeks of age) were superovulated by intraperitoneal injection of 10 IU of pregnant mare serum gonadotropin (PMSG) (Novormon®, Laboratorios Syntex S.A. Argentina), and 48 h later, with 10 IU of human chorionic gonadotropin (hCG) (Laboratorios Serono®). Females were then paired with males of proven fertility. The morning in which vaginal plugs were observed was counted as the first day of pregnancy.

Embryo collection

Preimplantation embryos at morula stages were recovered in Ham-F10 culture medium supplemented with 0.4% of BSA from oviducts and uteri of pregnant females killed by cervical dislocation on day 3 of pregnancy (72 hours following hCG). Embryos were flushed from the oviducts and uterus using a 30-g needle on a 1-cc syringe.

Culture media

To obtain conditioned media (CM) morulas were cultured in groups of 5 (CM5) or in groups of 10 (CM10) in microdrops (25µl) of Ham-F10 culture medium supplemented with 0.4% of BSA during 24 h and later they were removed. Ham-F10 media (supplemented with 0.4% of BSA) was used as control group.

Embryo culture and methods

Three hundred and sixty five (365) experimental embryos at morula stage were allocated randomly to CM5 media (n=115), CM10 media (n=105) and Ham-F10 media: (n=145) and cultured in groups of 5 (five)

| TABLE 1. Effect of conditioned media with embryos at morula stage on the percentage of differentiation in preimplantational mouse embryos. |
|---|---|---|---|
| culture media | embryos evaluated | percentage of differentiation |
| Ham-F10 (control) | 145 | 49.66 | 64.14<sup>a</sup> |
| CM5 | 115 | 53.04 | 80.00<sup>b</sup> |
| CM10 | 105 | 60.00 | 74.79<sup>ab</sup> |

CM5: conditioned media with five embryos at morula stage. CM10: conditioned media with ten embryos at morula stage.

<sup>a-b</sup>: values with different letter within the same column were significantly different (p<0.05)
for 96 h at 37°C under silicon oil (Squibb) in a humidified atmosphere of 5% CO₂ / 95% air. Morulas were evaluated after culture periods of 24 and 48 h (96 and 120 h post hCG, respectively) to study differentiation rates. To evaluate hatching rate, the embryos were scored after 48, 72, 78 and 96 h of culture (120, 144, 150 and 168 h post hCG).

Statistical analysis

Percentages of cell differentiation (number of embryos which reached the blastocyst stage / number of cultured embryos at the morula stage per 100) and hatching (number of embryos developed to the hatched blastocyst stage / number of cultured embryos at the morula stage per 100) were analyzed by the software PROC CATMOD Procedure of SAS (SAS Institute Inc.), 1989. The significance level accepted for differences among culture conditions was established at P<0.05.

Results

Our results show that after 24 h of culture no significant differences in blastocyst formation could be found between the Ham-F10 medium (control group) and conditioned medium with five or ten embryos. However, after a 48 h culture period conditioned medium CM5 increased the differentiation rate compared with the Ham-F10 medium (P<0.05; Table 1). Conditioned medium CM10 did not show differences respect to Ham-F10 medium.

The hatching rate was similar to Ham-F10 medium value for CM5 or CM10 media at 48 h of culture (27.59%, 23.47% and 19.05% respectively) (Figure 1). When this parameter was analyzed at 78 and 96 h of culture, we observed that the CM5 medium caused a significant increase compared to Ham-F10 medium (66.96 vs. 52.41% and 70.43 vs. 55.17%, respectively) (P<0.05; Figure 1). After 72, 78 and 96 h of culture the number of hatching blastocysts for embryos cultured in CM10 medium was significantly higher than that from Ham-F10 medium (64.76 vs. 47.59%, 67.62 vs. 52.41% and 73.33 vs. 55.17%, respectively) (P<0.05; Fig. 1).

Discussion

The effect of conditioned media observed in this work has a close relation to two phenomena that occur during embryo development. On one hand, preimplantational embryos themselves synthesize autocrine growth factors and their receptors (Rappolee et al., 1988; Heyner et al., 1989; Wiley et al., 1992; Terada et al., 1997; Markham and Kaye, 2003). On the other hand, in vitro developed embryos show coopera-

Figure 1: Effect of conditioned media with embryos at morula stage on the percentage of blastocysts hatching in preimplantational mouse embryos.
Control: Ham-F10 media. CM5: conditioned media with five embryos at morula stage. CM10: conditioned media with ten embryos at morula stage. Different letters show significant differences (p<0.05)
tive interaction where the rate of embryo development is density-dependent (Wiley et al., 1986; Paria and Dey, 1990; Lane and Gardner, 1992; Teruel and Smith, 1997; O’Neill, 1997; Rijnders and Jansen, 1999).

Our model of study with conditioned media by the embryos itself confirms the hypothesis about an autoregulation during preimplantational development. The results of this work suggest that mouse embryos at morula stage cultured for 24 h produce factor or factors that modify the culture media and affect the in vitro preimplantational development.

The effect on differentiation rate observed with CM5 or CM10 media confirms that the blastocyst formation is a process modified by embryo factors acting in an autocrine manner. The presence of receptors for different growth factors in embryos at preimplantation stage could probably explain the response observed. The fact that CM5 medium has been the one that increased differentiation compared with Ham-F10 medium instead of CM10 medium could be a consequence of the concentration of factors produced by five embryos being the optimal to achieve the morula to blastocyst transformation. Future works are needed to identify the factor or factors produced by the embryos and their concentration at 24-48 h of culture to determine the different effect of CM5 and CM10 media.

With respect to hatching rate, our results show that from 78 h of culture this parameter increased indistinctly in response to CM5 or CM10 compared to Ham-F10 media showing an effect of factors released by embryos to the culture media. This indicates that for our proposed model of conditioned media, from 78 h of culture, although the culture increased it did not do it in relation to the number of cultured embryos. Nevertheless, the fact that a beneficial effect was observed agrees with previous reports from essays in which, although conditioned media was not used as in the model proposed here, embryos were cultured in groups and an increase in such a parameter was observed. The increased hatching observed after the conditioned media were used becomes interesting considering the biological importance of this process. Further works should determine the reasons why no differences were found in the response after culturing embryos in CM5 or CM10 media for 78 h in culture, probably different concentrations of trophic factors achieved during 24 h for both media are not enough to modify the final hatching rate. These results suggest that differentiation and hatching would be differently regulated processes.

In conclusion, the proposed model of conditioned media confirms that endogenously produced factor or factors enhance the mouse embryo development in vitro acting as autocrine embryotrophic factors.

References


