ANIMAL EXPERIMENTATION

Globulins in Protein Supplements Promote the Development of Preimplantation Embryos

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Submitted: April 29, 1999

Accepted: May 27, 1999

Purpose: Our purpose was to investigate the effect of α - and β -globulins contained in protein supplements on the development of preimplantation embryos.

Methods: Mouse one-cell embryos were cultured in medium supplemented with 4 mg/ml human serum albumin (HSA), 4 mg/ml HSA plus human globulins (0.2, 0.4, 0.8, and 1.6 mg/ml) that consisted predominantly of α - and β -globulins, or 10% Plasmanate Cutter (PC). Blastocysts developed in media supplemented with these various protein sources were stained with Hoechst 33342 to determine the number of cells.

Results: Supplementation with 0.4 to 1.6 mg/ml globulins or PC significantly increased the rate of blastocyst development compared with that observed with the addition of HSA. Supplementation with globulins significantly increased the hatching rate in a dose-dependent manner. The number of cells in the blastocysts was significantly increased when the embryos were cultured with 0.8 mg/ml of the globulins or PC.

Conclusions: The present observations suggest that α - and β -globulins in protein supplements promote embryo development and hatching.

KEY WORDS: globulin; protein supplement; embryo development.

INTRODUCTION

Establishing optimal culture conditions for human preimplantation embryos is important to yield a high pregnancy rate in human in vitro fertilization and embryo

transfer (IVF-ET) programs. The culture media used in human IVF are supplemented with a protein source because it is believed that supplementation with protein supports embryo development and reduces potential toxicity in the culture environment (1,2).

Recent studies reported an excellent development rate to blastocysts and pregnancy rate using a serum substitute in human IVF, such as Synthetic Serum Substitute, Plasmatein, and Plasmanate (3–7). These serum substitutes contain α - and β -globulins in 12–17% of the total protein content. It is suggested that α - and β -globulins in the serum substitutes exert a growth-promoting effect on embryo development. However, this effect has not been fully elucidated because no attempt has been made to examine how different concentrations of globulins affect embryo development. Therefore, to clarify the potential role of α - and β -globulins in protein supplements, we examined embryo development in media containing various concentrations of globulins.

MATERIALS AND METHODS

B6C3 F₁ female mice at 5–7 weeks of age (Shimizu Labo-Supplies Co. Ltd., Kyoto, Japan) were superovulated with an intraperitoneal injection of 7.5 IU of pregnant mare serum gonadotropin (PEAMEX; San-kyo Co., Tokyo), followed 48 hr later by 7.5 IU of human chorionic gonadotropin (hCG; Pregnyl; N.V. Organon, Tokyo). The female mice were placed overnight with male mice of proven fertility, then inspected for vaginal plugs the following morning. The females were killed by cervical dislocation 20 hr after the injection.

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tion of hCG, and one-cell embryos were collected from the oviducts. The one-cell embryos were cultured in human tubal fluid (HTF; Irvine Scientific, Santa Ana, CA) supplemented with 4 mg/ml HSA (Sigma Chemical Co., St. Louis, MO) or 4 mg/ml HSA plus human globulins (0.2, 0.4, 0.8, and 1.6 mg/ml) (Cohn Fraction IV-4; Sigma Chemical Co.) which contained 54% α -globulin, 35% β -globulin, and 11% albumin. Embryos were also cultured supplemented with 10% Plasmanate Cutter (PC; Bayer Co., NC), which contains 12% α - and β -globulins and 88% albumin. More than one hundred embryos were cultured in medium that was supplemented with each of the protein supplements. Embryo development was observed under a dissecting microscope every 24 hr, and the development rate to blastocysts (after 96 hr) and hatched blastocysts (after 120 hr) was calculated. The blastocysts developed in the medium supplemented with 4 mg/ml HSA, 4 mg/ml HSA, plus 0.8 mg/ml human globulins or 10% PC were stained with Hoechst 33342 (Eastman Kodak, Rochester, NY), and the number of cells in the blastocysts was calculated. All experiments were performed in accordance with the *Guiding Principles for the Care and Use of Research Animals* promulgated by the Society for the Study of Reproduction.

RESULTS

The rate of blastocyst development in the medium supplemented with 0.4 to 1.6 mg/ml of globulins plus HSA was significantly higher than that in the medium supplemented with HSA (Fig. 1). The rate of hatching in the medium supplemented with 0.2 to 1.6 mg/ml of globulins plus HSA was also significantly higher than that in the medium supplemented with HSA (Fig. 1). Supplementation with globulins increased the rate of hatching in a dose-dependent manner. The number of cells in blastocysts developed in the medium supplemented with 0.8 mg/ml globulins plus HSA was significantly higher than in that supplemented with HSA alone (24.5 ± 3.4 vs 21.7 ± 2.8 ; $P < 0.01$). The development rate to blastocysts and hatched blastocysts in the medium supplemented with PC was significantly higher than in the medium supplemented with HSA alone ($P < 0.01$) (Fig. 1). The number of cells in blastocysts developed in the medium supplemented with PC was significantly higher than in that supplemented with HSA (28.7 ± 3.3 vs 21.7 ± 2.8 ; $P < 0.01$).

DISCUSSION

The present study demonstrates that globulins containing predominantly α - and β -globulins in protein supplements promote the development of mouse one-cell embryos to blastocysts and increase the number of cells in blastocysts. In particular, blastocyst hatching was promoted by the addition of globulins in a dose-dependent manner. The total protein concentration in the medium supplemented with HSA plus globulins was higher than in that supplemented with HSA alone. To rule out the possibility that a rich concentration of protein in HSA plus globulins may stimulate embryo development, embryos were cultured in medium supplemented with 5 or 6 mg/ml HSA. However, the growth-promoting effect could not be observed in the medium supplemented with high concentrations of HSA (data not shown). These results suggest that α - and β -globulins in the globulin fraction may act as a growth-promoting factor for embryo development and hatching.

Serum macromolecules added to embryo culture medium are thought to act as a chelating agent of toxic substances, to facilitate membrane stabilization, and to act as an antioxidant. Pool *et al.* suggested that adding a significant amount of polyhydroxy compounds comprising the α - and β -globulin fraction alters water dynamics and influences solvent properties to mimic the microenvironment surrounding embryos in vivo (4). Therefore, it is possible that supplementing α - and β -globulins improves the culture environment and enhances the developmental ability of mouse embryos.

The globulin fraction employed in this study is a crude fraction separated from human plasma that contains 54% α -globulin, 35% β -globulin, and 11% albumin. It is reported that the high molecular weight nonalbumin components of commercial bovine serum albumin promote hatching of rabbit blastocysts (8). Therefore, another possibility may be that the globulin fraction contains unknown growth-promoting factors that enhance blastocyst development and hatching of mouse embryos. Supplementation with globulins specifically stimulated blastocyst hatching, and similar stimulatory effects have been observed by adding growth factors to the culture medium. We have reported that EGF and TGF- α stimulated blastocoel expansion of preimplantation mouse embryos (9). These results suggest that growth factors may be one possible substance contained in the globulin fraction. In the present study, the hatching rate and the number of cells in the blastocysts were most pronounced in

Fig. 1. Effect of supplementation with globulins on the development of mouse one-cell embryos to blastocysts and hatched blastocysts. The development rates to blastocysts and hatched blastocysts in the medium supplemented with HSA were 70% and 8%, respectively. The development rates to blastocysts in the medium supplemented with HSA plus 0.2, 0.4, 0.8, and 1.6 mg/ml globulins were 76, 88, 86, and 84%, respectively. The development rates to hatched blastocysts in the medium supplemented with HSA plus 0.2, 0.4, 0.8, and 1.6 mg/ml globulins were 16, 19, 25, and 38%, respectively. The development rates to blastocysts and hatched blastocysts in the medium supplemented with 10% PC were 86 and 55%, respectively. * $P < 0.01$ compared with HSA. ** $P < 0.05$ compared with HSA.

the medium supplemented with PC, suggesting that PC contains growth-promoting factors other than those contained in globulin fraction.

Using an *in vitro* mouse embryo culture model, we demonstrated that supplementation with globulins promotes embryo development and hatching. These results support the use of globulin-containing protein source for culture media in human IVF.

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