CLINICAL ASSISTED REPRODUCTION

Prolonged Culture of Human Cryopreserved Embryos with Recombinant Human Leukemia Inhibitory Factor

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Purpose: To evaluate the efficiency of recombinant human leukemia inhibitory factor (LIF) in the prolonged culture of human cryopreserved-thawing embryos.

Methods: After thawing, all embryos were divided into four groups: (1) Human tubal fluid (HTF), (2) HTF + LIF, (3) M3TH medium, and (4) M3TH medium plus LIF. Following prolonged culture, embryo development in each group was compared.

Results: In embryo development from about the 2- to 4-cell to 9- to 16-cell stage, there were nonsignificant differences between each group. There was lower morula formation rate in group 1 (6.9%) than those in other groups (23.2%, 19.7%, 23.1%). The lower blastocyst formation in group 1 and 3 (0%, 0%) than those in group 2 and 4 (11.0%, 12.8%) were noted.

Conclusions: LIF is beneficial for preimplantation embryos. LIF does not influence the early embryo development. LIF-supplemented HTF provided a similar culture environment for thawing embryos as LIF-supplemented M3TH medium.

KEY WORDS: Blastocyst; leukemia inhibitory factor; prolonged culture.

INTRODUCTION

For enhancing embryo development in vitro, several attempts have been made, including the application of

leukemia inhibitory factor (LIF) (1). LIF has been demonstrated to augment blastocyst formation and hatching in mouse (1,2) and bovine embryos (3) and subsequent implantation rates in sheep (4). However, the literature about the LIF application in the human embryo is scanty. Few investigators have ever demonstrated the application of LIF in the prolonged culture of human embryo.

Dunglison *et al.* (5) demonstrated that LIF could significantly enhance the blastocyst formation of human embryos in a serum-free medium. Jurisicova *et al.* (6) demonstrated that LIF does not enhance the development of early stage human embryos. No other investigators have ever demonstrated the human embryo application of LIF in prolonged culture in a large series. In this series, using the different media [human tubal fluid (HTF) or M3TH] with the supplement of LIF or not, we attempted to compare the effect of LIF on the early and later development of human embryos.

MATERIALS AND METHODS

All cryopreserved human embryos were donated by patients who accepted a successful in vitro fertilization (IVF) procedure and resulted in a live birth between January 1991 and December 1996. The protocol was approved by the Ethical Committee of the China Medical College Hospital. Informed consent was signed by all couples who donated their embryos. All embryos were recovered after controlled ovarian stimulation with gonadotropin [follicle-stimulating hormone (FSH), Metrodin, Serono, Rome, Italy; human meno-

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pausal gonadotropin (hMG), Pergonal, Serono, Rome, Italy] and with or without the long or short protocol down-regulation of the gonadotropin-releasing hormone agonist suppression (Leuprolide acetate depot, 1.875 mg subcutaneous single dosage, Takeda Chemical Industries, Ltd, Japan; Leuprolide acetate, 1 mg/day, Abbott Laboratories, Chicago, IL; Supermon, 0.5 mg $\times$ 3 times/day, Hoechst AG Frankfurt am Main Germany) (7).

After four or five embryos were transferred, the other embryos were cryopreserved with the ultrarapid method. All cryopreserved embryos were thawed by the ultrarapid method (8,9). After the thawing procedure, all embryos were evaluated and their cell number and grade were recorded, using a light microscope. All the thawing embryos with poor quality (grade IV) or abnormal division (1 cell, 2 pronuclear) were excluded from this series.

All embryos were divided randomly; embryos from a single patient were divided equally into four groups with different culture mediums: (1) 30 μ l microdroplets of HTF medium containing 0.5% human serum albumin (HSA); (2) 30 μ l microdroplets of HTF containing 0.5% HSA and LIF (1000 IU/ml); (3) 30 μ l microdroplets of M3TH medium (Medi-Cult, Inc., Hopkinton, MA); and (4) 30 μ l microdroplets of M3TH medium containing LIF (1000 IU/ml). The LIF was prepared as the procedure of Chang *et al.* (10). All cleaved embryos were transferred into a dish containing the medium and covered with oil. Each embryo was cultured separately in its own well, making evaluation of embryo development easy. All embryos were then incubated in an incubator at 37°C and 5% CO₂ humidified atmosphere.

In the following 5 days, all embryos were evaluated and their further division and development recorded. The microdrop media of all embryos were replaced everyday. Embryonic development was evaluated by

morphological observation under a light microscope everyday. The number and percentage of embryos reaching the 5- to 8-cell, 9- to 16-cell, morula, and blastocyst stages were recorded at 24, 48, 72, 96, and 120 hr postembryo thawing.

Further evaluations of the embryo cleavage, development, and blastocyst formation in different groups were made. The roles of LIF and different media in the prolonged embryo culture were evaluated. The further statistic analyses were performed by using the SAS statistical package. The χ^2 test and logistic regression model test were used for statistic analyses. A *P* value of < 0.05 was considered statistically significant.

RESULTS

After thawing, the embryos in all groups were statistically comparable in their qualities (including the grade and cell numbers). In comparing the early embryo development from 2- to 4-cell to 9- to 16-cell stage, there were nonsignificant differences between each group (Table I). In morula development, there was lower morula formation rate in group 1 (6.9%) than those in other groups. The nonsignificant difference in morula formation rate between groups 2, 3, and 4 were noted (23.2%, 19.7%, 23.1%, respectively) (Table I). In blastocyst formation, the higher blastocyst formation rates in groups 2 and 4 (11.0%, 12.8%) than in 1 and 3 (0%, 0%) were noted. There were nonsignificant differences between groups 2 and 4 in blastocyst formation.

DISCUSSION

Currently, most authors agreed as to the benefit of LIF on preimplantation stages of embryos (11,12). We

Table I. Embryo Development of Four Groups After Prolonged Culture

	2- to 4-Cell embryo	5- to 8-Cell embryo ^a	9- to 16-Cell embryo ^a	Morula ^b	Blastocyst ^c
Group 1 (HTF) ^d	72	31 (43.1%)	26 (36.1%)	5 (6.9%)	0
Group 2 (HTF + rhLIF) ^e	82	40 (48.8%)	33 (40.2%)	19 (23.2%)	9 (11.0%)
Group 3 (M3) ^d	76	34 (44.7%)	29 (38.2%)	15 (19.7%)	0
Group 4 (M3 + rhLIF) ^e	78	36 (46.2%)	32 (41.0%)	18 (23.1%)	10 (12.8%)

^a Nonsignificant differences in the early embryo development (2- to 4-cell to 9- to 16-cell stages) between four groups.

^b Lower morula development rate in group 1 compared with other groups ($P < 0.05$).

^c Lower blastocyst development rate in group 1 and 3 compared with groups 2 and 4 ($P < 0.05$).

^d Nonsignificant differences between groups 1 and 3 between 2- to 4-cell and morula stages; HTF, human tubal fluid.

^e Nonsignificant differences between groups 2 and 4 between 2- to 4-cell and blastocyst stages; rhLIF, recombinant human leukemia inhibitory factor.

have demonstrated that the LIF enhanced the embryo development in the preimplantative stages of mouse embryos (1). The main controversy exists in the role of LIF on the early stage of embryo development. Michell *et al.* (13) demonstrated that LIF enhances embryo development and decreases embryo fragmentation even in the 2-cell stage. In contrast, Jurisicova *et al.* (6) demonstrated that LIF in standard medium does not enhance the early stage development of human embryos. In this series, we also observed the nonsignificant influence of the LIF on the early embryo development.

Jurisicova *et al.* (6) demonstrated that LIF does not enhance the expanded blastocyst development of human embryos. However, our result revealed the beneficial effects of LIF on the development of the preimplantative embryos. This might be due to the different embryo origin (fresh or cryopreserved embryos). In this series, the embryo development was suboptimal. This may be due to the formation of the ice crystal during the cryopreserving–thawing process, which may damage the embryos. We also observed that LIF-supplemented HTF could supply similar environment as compared to the LIF-supplemented M3TH medium.

Many authors have demonstrated that the beneficial effects of coculture may be due to LIF production by feeder cells (14). It has been demonstrated that LIF was secreted into the culture media by Vero cells and fetal fibroblasts (2). In this current series, we used the recombinant LIF instead of the LIF released from the coculture cells for the prolonged embryo culture. The advantage of recombinant LIF over the LIF released from the coculture cell is its convenience, commercial availability, and the absence of the risk of virus infection. It provides a safer and cell-free approach to promote the human embryos development in vitro.

It has been demonstrated that the customary culture media lack important regulators for prolonging embryo development (15,16). Conventional embryo culture media, such as Earle's (17) and NTF (18), do not support high levels of embryo development to the blastocyst stage. Jones *et al.* (19) described the serum-free, cell-free culture media (Gardner's G1 and G2 medium) for the development of up to 52% of all zygotes to the blastocyst stage in vitro. Since customary culture media used in in vitro fertilization are not suitable for the prolonged embryo culture, in this series, we tried the more complex and nutritive media (M3TM medium) for extending the culture time (20,21). The LIF-supplemented M3TM medium is better than the simple M3TH medium in the preimplantative embryo development.

The influence of HSA on the embryo development is controversial. Dunglison *et al.* (5) demonstrated that serum is not necessary for human embryo development in vitro. Gardner *et al.* (22) demonstrated that a serum-free chemically defined medium could result in the better blastocyst formation of the embryo than the complex tissue culture medium (Ham's F-10) with serum. In this series, for the embryo number limitation, we could not further compare either the supplement or the HSA. Because the content of M3 includes HSA, we added the HSA in the media of groups 1 and 2 to decrease the influence of HSA in human embryo development and further compared the HTF and M3 culture medium.

In conclusion, LIF has positive effects on preimplantation embryo development. LIF does not provide the stimulation upon early embryo development. The LIF-supplemented HTF medium could provide a similar prolonged culture environment for thawing embryos as the LIF-supplement M3TH medium. However, a larger series and multicenter cooperation are required to evaluate the effects of LIF and different culture medium in the prolonged culture of human embryos.

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