THE IN VITRO FERTILIZATION RATE OF MOUSE OVA IN THE ABSENCE OR PRESENCE OF RECOMBINANT HUMAN LEUKEMIA INHIBITORY FACTOR

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ABSTRACT

Objective: To examine the effect of human recombinant leukemia inhibitory factor in different doses on rate of fertilization of mouse ova.

Design: Prospective study.

Setting: Department of Anatomy, laboratory of cell culture.

Animals: Female NMRI mice 6 to 8 weeks old.

Interventions: Mice were killed at 12-14 hours after hCG or 36-38 hours after hMG injection. Mature oocytes were obtained and divided randomly into 5 groups. Oocytes in group A (n=157) were cultured as the control group in TYH medium. Oocytes in groups B, C, D, E (n=137, 154, 166 and 159, respectively) were cultured in the same medium supplemented with recombinant human leukemia inhibitory factor in four different concentrations (5, 7.5, 10, 20ng/ml, respectively) for 1 hour. After that time 100000 spermatozoa were added to every drop and after 24-26 hours two cell embryos were recorded. Fertilization was assessed by recording the number of 2-cell embryos and analysed by X^2 tests.

Main outcome measurment: Two cell embryos.

Results and Conclusion: No significant difference was detected in the rate of two cell embryos in the studied experimental groups as compared with the rate of two cell embryos in control group (Group A). This study showed that, different concentrations of recombinant human of leukemia inhibitory factor in standard medium does not enhance in vitro fertilization rate of mouse oocytes.

KEY WORDS: Recombinant human leukemia inhibitory factor (rhLIF), Fertilization, Two cell embryo, NMRI mice.

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INTRODUCTION

Leukemia inhibitory factor was first identified in 1986 by Metcalf and his colleagues at Walter and Eliza Hall institute of Medical Research in Melborne, Australia.[1]

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LIF is a secreted glycoprotein with a range of molecular weight forms, from 38 to 67 Kd, resulting from differential glycosylation of protein of approximately 20 Kd[2]. LIF has the four á helix cytokine structure that contains six cysteine residues, already known to occur in many hematopoietic factors.[3,4]

Leukemia inhibitory factor plays an important role in the embryo development.[5] LIF has been shown to enhance in vitro blastocyst development in mice[6,7] in vitro blastocyst hatching in sheep and increased pregnancy rates for in vitro cultured embryos transferred back into recipient ewes.[8]

It has been reported that the expression of LIF in the endometrium is absolutely essential for mouse embryo implantation.[6,9] In humans the peak expression of LIF and its receptor (LIF-R) in the endometrium at the mid

secretory phase of the menstrual cycle also suggests a potential autocrine role for LIF in regulatory human implantation.[10] However, the target of LIF may not be restricted to the endometrium, because there is a report revealing the expression of LIF in the epithelium of ampullary portion of fallopian tube.[11] Because the fertilization takes place in the ampullary portion of fallopian tube and LIF was expressed in the fallopian tube, suggesting that LIF may play a role in fertilization.

Our objective was to determine the effect of different concentrations of recombinant human leukemia inhibitory factor on the rate of mouse oocyte fertilization.

MATERIALS AND METHODS

Oocyte collection: Female NMRI mice that were between 6 to 8 weeks old were administered intraperitoneally with 10 IU Human Menopausal Gonadotropine (HMG) for superovulation, this was followed 46-48 hours later by the intraperitoneal administration of 10 IU Human Chorionic Gonadotropine (HCG).

Mice were killed 12-14 hours after hCG injection by cervical dislocation method. After disinfection with 70% alcohol and opening the abdomen wall, the Y shaped uterus, ovaries and oviducts were identified. The oviducts were excised as follows: clamping cornuas, dissecting the peritoneum and fat between ovary and tube and then cutting the fallopian tube from the proximal end and cumulus-oocyte complexes were collected in TYH medium. The granulosa cells of oocytes were removed by pipetting in TYH medium containing 80IU/ml hyaluronidase and mature oocytes obtained and randomly divided into 5 groups. Oocytes in Group A (n=157) were cultured as the control group in TYH and oocytes in Group B, C, D and E (n=137, 154, 166 and159) were cultured in the same medium supplemented with recombinant human leukemia inhibitory factor (Sigma, L-5158) in four different concentrations (5, 7.5, 10, 20ng/ml) for 1 hours in atmosphere of 5% CO2 in air at 37Ú C.

Table-I: Composition (Mm/L) of TYH and KSOM.

	TYH	*KSOM*
NaCl	119.3	95
KCl	4.7	2.5
CaCl2	1.71	1.71
KH2Po4	1.2	0.35
MgSo4.7H20	1.2	0.2
NaHCo3	25.1	25
Glucose	5.56	0.2
EDTA-2NA	—	0.01
Na pyruvate	1	0.2
Glutamine	—	1
BSA(fraction V)	4mg/ml	1mg/ml
Streptomycin	0.05mg/ml	0.05mg/ml
Penicillin G(k-salt)	100Iu/ml	100Iu/ml

Sperm preparation: Males of proven fertility were killed and the cauda epididymis immediately removed to a 150ìl drop of TYH medium under mineral oil (Sig., embryo-tested, cat#M8410). The epididymis contents were squeezed out and the proceed for 1-2 hours at 37ÚC in 5% in humidified air. Sperm concentration was determined with haemocytometer.

Fertilization in vitro: Fertilization in vitro was carried out in 1 ml drops of TYH medium under mineral oil. A preincubated capacitated sperm suspension was gently added to the freshly ovulated ova to give a final motile sperm concentration on 100000/ml.

The combined sperm oocyte suspension was incubated for 4-6 hour. The ova were then washed through several changes of medium and finally incubated in 50ìl drops of KSOM without or with different concentrations of LIF (5, 7.5, 10 and 20ng/ml) under mineral oil. Fertilization was assessed by recording the number of 2 cell embryos 24-26 hour after completion of fertilization in vitro.

Culture media: Modified KRB (12) called TYH (Table-I) without LIF was used for sperm preincubation and TYH medium with different concentrations (0, 5, 7.5, 10 and 20ng/ml) of LIF for IVF. Pyruvate and LIF was added immediately after overnight equilibration of the medium. Invitro fertilized zygotes were cultured in KSOM (Table-I) suplemented with MEM essential amino acid solution (Sig. M-5550) (10ìl/ml) and MEM non-essential amino acid solution (Sig. M-7145) (5ìl/ml). Glutamine (Sig. G-5440), pyruvate (Sig. P-5280), BSA and aminoacid solution and LIF were added.

Table II: The number of ova exposed to spermatozoa (IVF) and zygotes that cleaved to the 2-cell stage after 24-26 h in culture in 5 variants of KSOM.

2 cell embryo	*No. of ova*	*Groups*
83.43%(131)	157	KSOM
84.67%(116)	137	KSOM + 5 ng/ml hrLIF
86.36% (133)	154	KSOM + 7.5 ng/ml hrLIF
85.54% (142)	166	KSOM + 10 ng/ml hrLIF
83.64% (133)	159	KSOM + 20 ng/ml hrLIF

Statical analysis: X^2 tests were used to compare the five groups. A difference of $p<0.05$ was considered to be significant.

RESULTS

Table-II summerizes the precentage of ova that cleaved to the 2 cell stage after 24-26 hour culture in the 5 media.There was non stimulatory and non-inhibitory effect of rhLIF when added to culture medium on rate of fertilization of mouse oocytes. The precentage of mouse ova reaching the stage of 2-cell stage in each groups (A=83.43%, B=84.67%, C=86.36%, D=85.54%, E=83.64%) were non significantly different.

DISCUSSION

The fallopian tube is a complex but delicate organ that performs numerous functions. It has been suggested that oviduct fluid and oviduct epithelium modulate maturation and transport of gametes and embryos.[13] In the fallopian tube high constitutive levels of LIF were expressed in the ampullary section suggesting that LIF may play a role during early embryonic development or on the fertilization.[11] It is possible that the beneficial effects of coculture are due to LIF production by the feeder cell[14] but in this current series, we preferred to use the recombinant human LIF instead of LIF released from the coculture cells for invitro fertilization. The advantage of recombinant LIF over the LIF released from the coculture cell is its convenience, commerical availability and the absence of the risk of virus infection.[15] There is controversy as to the effect of LIF on the different development stages of embryo development. Michell et al[7] demonstrated that rhLIF enhances blastocyst formation and decreases embryo fragmentation even in the two cell stage. In contrast, Jurisicova et al[16] demonstrated that rhLIF in standard medium does not enhance the development of early stage human embryos. Tsai et al[17] demostrated that LIF has positive effect on preimplantation embryo (morula, blastocyst and hatching) development and has no significant influence on early embryo (2-16 cell stage) development. Why the LIF is secreted from ampullary portion of fallopian tube? The answer to this question needs more study? As we know there is no report about the effect of LIF on rate of fertilization. The present study is unique study in showing the effect of LIF on in vitro fertilization rate of mouse ova. This series provides information about the effect of different concentrations of LIF on the rate of fertilization. There was non stimulatory effect of LIF where it was added to the medium. LIF modulate sperm development and plays a role in the survival of sertoli cells and gonocytes in vitro.[18-20]

It has been shown that incubation of spermatozoa in the media with LIF results in greater sperm motility and survival rates. Attar et al[21] demonstrated that effect of LIF on sperm motility and survival was concentration-dependent and significatly higher after 24 and 48 hours exposure, respectively and also LIF showed its maximal effect on sperm motility at a concentration of 5ng/ml wherase sperm survival was enhanced at higher concentrations. The maximal effect on sperm survival was reached at a concentration of 10ng/ml. The biological actions of LIF on sperm cells must be mediated through interactions with specific cellular receptors.Expression of LIF receptor mRNA was found in rat elongating spermatids and all mouse germ cells.[22] The demonstration of LIF receptors on these cells suggest that the effect of LIF is mediated by direct interactions with the spermatid and germ cells rather than by the indirect release of secondary substances. However, it seems neccessary to investigate further on this issue; for instance before doing the IVF, sperms could be incubated into the drops of TYH medium with

differrent concentrations of leukemia inhibitory factor and also in different ranges of time.

CONCLUSION

This study showed that different concentrations of recombinant human leukemia inhibitory factor in standard medium do not enhance in vitro fertilization rate of mouse oocytes. It is possible that recombinant human leukemia inhibitory factor in ampullary portion of fallopian tube may play a significant role in enhancing sperm activity or viability.

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