

Action of leukemia inhibition factor on in vitro maturation, fertilization and blastocyst development of Rabbit oocyte

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Abstract

Action of

leukaemia inhibition factor (LIF) on oocyte maturation, fertilization and blastocyst development of rabbit oocytes were studied in vitro

with 200 oocytes each in different concentrations of LIF in three experimental groups. LIF when added in the standard maturation medium increased the maturation rate significantly ($p < .000$). LIF also increased the fertilization rate significantly ($p < .006$) when added in the fertilization medium. The number of embryos reaching blastocyst stage was increased significantly on adding 100ng/mL of LIF in the embryo development medium. But the number of embryos reaching the hatching stage was decreased significantly ($p < .05$) on adding LIF in the embryo development medium.

Abbreviations: In vitro maturation (IVM), In vitro fertilization (IVF), LIF- Leukaemia inhibitory factor

INTRODUCTION

Leukaemia inhibitory factor (LIF) is a polyfunctional glycoprotein cytokine whose inducible production can occur in many, perhaps all, tissues. (Kuriek J, 2000). LIF is essential for blastocyst implantation and the normal development of hippocampal and olfactory receptor neurons. LIF is used extensively in experimental biology because of its key ability to induce embryonic stem cells to retain their totipotentiality. Leukaemia inhibitory factor (LIF) has been shown to be essential for the implantation of mouse blastocysts (Zeng-Ming yang et al., 1998). Mouse embryonic stem (ES) cells can be differentiated in vitro into near homogeneous populations of both neurons and skeletal muscle as well as other cell types (Dinsmore et al., 1998). In vitro maturation, fertilization and embryo development were successfully reported in several mammalian species such as cattle, sheep and buffalo, etc (Wang S et al, 1998, Totey et al., 1992). A large number of experiments were conducted for in vitro standardization of oocyte maturation, sperm capacitation, fertilization, and embryo development in different species. Protein supplementation during in vitro

maturation can profoundly affect both the rate and overall efficiency of the maturation procedure. Follicular fluid originating from competent follicles increased the developmental competence of abattoir-derived oocytes (Atef Ali et al 2004). There are different factors like concentration of hormones, serum, capacitating agents etc. which affects the fertilization rate of the spermatozoa. Oocytes fertilized by spermatozoa incubated in 20 ng/ml nor epinephrine fertilize earlier in vitro than sperm pre-incubated in medium alone, and provide additional support for the role of nor epinephrine in sperm capacitation and the acrosome reaction (Way et al., 2005).

Rabbit oviduct epithelium cell monolayers were able to support the development of cleaved bovine embryos to the blastocyst stage as compared with the embryos on cow oviduct epithelium cell monolayers (Prokofieve et al 2005). When added at 120 hours post insemination, glucose improved development to the blastocyst and expanded blastocyst stages, compared with no glucose (Pavasuthipaisit K et al., 1992).

Stimulation of bovine IVM/IVF embryo development by somatic cell conditioning is due to removal of inhibitory

influences from the culture environment (Barry et al., 1992).

In the present investigation effect of LIF in the maturation, fertilization, development of rabbit oocytes were studied. We have investigated the effect of LIF on gametes in vitro, on the hypothesis that the ovulatory peak of LIF can exert an influence on gametes present within the oviduct. We also investigated the effect of LIF on In vitro fertilization and embryo development in oocytes from rabbit.

MATERIALS AND METHODS

Chemicals were purchased from M/s. Hi media, Mumbai. LH and FSH were procured from Sigma Aldrich, Bangalore.

METHODS

COLLECTION OF OOCYTES AND MATURATION

Oocytes were collected from rabbit ovaries obtained from the slaughter house daily. It was brought to the laboratory within 2-3 hrs. of slaughter in a thermo flask with warm water at 30-35 °C supplemented with 50 µg/ml of gentamycin. Ovaries were crushed and the follicular fluid was collected in a 50ml sterile conical tube and kept at 38 °C for 10-15 minutes to settle down the debris. Then spin at 500 rpm for 5 min. The supernatant was then discarded and the pellet was carefully diluted with TL medium buffered with 10 mM HEPES and low bicarbonate TALP (Parish JJ, 1998) which was pre incubated at 38°C in CO₂ incubator for 4-5 hrs. It is then screened under microscope for oocytes. The mature oocytes are seen having compact cumulus cells and clear cytoplasm. The number of good quality oocytes collected was counted. The collected oocytes are then thoroughly washed in medium with TCM 199+HEPES and 10% fetal calf serum, 0.2 mM pyruvate and 50 µg/ml of gentamycin and randomly assigned to experimental groups. Oocytes were kept in maturation drops as 20 oocytes per 50 µl drop and layered with paraffin oil which was kept in CO₂ incubator for equilibration. Maturation media consists of TCM 199 supplemented with sodium bicarbonate, 10 %FCS, 5 µg/ml FSH, 50 µg/ml LH, LIF and 0.2mg/ml gentamicin. These oocytes were again kept in CO₂ incubator at 38°C for 18-24 h for maturation.

The experiment was repeated for different concentrations of LIF such as 0, 5 and 10 µg/ml in the maturation medium. Total 200 numbers of oocytes were taken for each concentration of LIF and the experiments were done in triplicate.

IN VITRO FERTILIZATION

Frozen thawed semen collected from Kerala Live Stocks department was used for in vitro fertilization.

One straw of semen of a particular male is taken out from liquid nitrogen and transferred to a water bath at 38° C. Dead and live sperm were separated by percoll separation. The pellet was then diluted to a required concentration of 2 x 10⁶ sperms /ml with TALP with HEPES (Bavister et al., 1983, Fuki et al 1983, Niwa et al 1986). It was then incubated with 10 oocytes /50 µl of the fertilization drops. The fully matured oocytes were collected from maturation media, washed with fertilization media and kept in fertilization drops i.e. 10 oocytes/50 µl of fertilization drop. Then washed diluted semen is added and kept for fertilization at 38°C in a CO₂ incubator for 24 to 36 hrs. Fertilization was carried out in TALP-FERT supplemented with heparin, penicillamine, hypotaurine, epinephrine, LIF and BSA. Fertilized oocytes were either used for fixing or transferred to embryo development medium. The experiment was repeated for 0, 5, 10 µg/ml of LIF concentrations in the fertilization medium.

The oocytes were then taken and the cumulus cells were removed by repeated pipetting with a small bored pipette. Cumulus free oocytes were either mounted or transferred for embryo development. (Totey et al., 1992). Oocytes were classified as normal if it is fertilized for single sperm otherwise poly spermy if fertilized by multiple sperms. If there is contamination the oocytes get degenerated.

Fertilized oocytes were transferred to CR₂ development medium supplemented with 10%FCS(v/v),BSA, gentamicin and LIF as described by Rosekrans and First (1991). It was then observed for blastocyst development at every 24 hours for eight days. Then the blastocysts were observed for embryo hatching stage in the medium with and without LIF. The evaluation of embryos for blastocyst, hatching etc were made according to the International Embryo Transfer Society manual (Stringfellow 1999). In brief the embryos were observed under a phase contrast microscope. The number of cells were counted.

STATISTICAL ANALYSIS

The maturation, fertilization rate was observed for different LIF concentration. The same experiment is repeated in replicate and data was analyzed by chi test.

RESULTS

Oocytes cultured in the medium with LIF showed an increase in the maturation rate than the medium without LIF. (TABLE I). When LIF at a concentration of 5 ng/ml was added to the maturation medium the number of good quality oocytes were increased by 21.5 %. The percentage again increased by 22.5 % when the concentration of LIF in the maturation medium increased from 5 to 10 ng/mL. (Table.1)The number of good quality oocytes were significantly higher than the number of oocytes in the maturation medium without LIF. ($p<0.000$)

The fertilization rate also increased significantly when LIF at concentration of 10 ng/mL is added in the fertilization medium. ($p<0.006$). (TABLE II). The fertilization rate has increased by 11.5% when LIF at a concentration of 5ng/mL is added in the fertilization medium. The rate has again increased by 11.5 % when the concentration of LIF in the fertilization medium increased to 10ng/mL. An increase in fertilization rate has obtained only when the oocytes matured in medium with LIF is used for fertilization. But when the oocytes cultered in medium without LIF is used, the rate of fertilization increase was negligible. (Table III).

When embryos were transferred to development medium with LIF at a concentration of 50 ng/mL, the inner cell mass increased significantly for the first eight days. After eight days not much differences were observed. The number of embryos reaching the hatching stage was significantly reduced on adding LIF in the development medium.

Figure 1

TABLE I. EFFECT OF LIF ON RABBIT OOCYTE MATURATION

CONCENTRATION LIF (ng/mL)	OF	No. of oocytes cultured	No. of good quality oocytes after 18 hours of culture	% Maturation	P value
0		200	85	42.5 ⁺ 5 ^a	
5		200	108	54 ⁺ 4.5 ^b	0.001
10		200	131	65.5 ⁺ 6.2 ^b	0.000

^{a,b} - significantly different.

Figure 2

TABLE II.EFFECT OF LIF ON FERTILIZATION RATE

CONCENTRATION LIF (ng/ml)	OF	No. of oocytes transferred for fertilization	No Of oocytes fertilized	%Fertilization	P value
0		200	42	21 ⁺ 7.1 ^a	
5		200	85	42.5 ⁺ 5.8 ^b	0.005
10		200	112	56 ⁺ 6.3 ^b	0.006

^{a,b} - significantly different.

Figure 3

TABLE III.COMPARISON OF FERITILZATION RATE OF OOCYTES MATURED AT DIFFERENT CONCENTRATION OF LIF IN THE MATURATION MEDIUM.

Oocytes collected from the medium matured at LIF con.	Concentration of LIF in the fertilization medium	No. of oocytes transferred	No. of oocytes fertilized	No. of oocytes not fertilized	No. of oocytes degenerated	%Fertilization	No of oocyte developed into blastocyst	No of oocyte developed to hatching stage
0	0	28	6	18	4	21.4 ⁺ 3 ^a	2	8
	5	28	8	10	5	28.5 ⁺ 2.5 ^a	7	
	10	28	7	15	4	25 ⁺ 5.2 ^a	7	
							pvalue<1.1	
5	0	36	15	19	2	41.6 ⁺ 6.1 ^a	14	2
	5	36	18	14	4	50 ⁺ 7.2 ^a	18	
	10	36	17	14	5	47.2 ⁺ 7.3 ^a	17	
							pvalue<0.05	
10	0	43	25	15	3	58.1 ⁺ 8 ^a	22	1
	5	43	27	14	2	62 ⁺ 7.5 ^a	26	
	10	43	28	20	1	64 ⁺ 4.8 ^b	28	
							pvalue<0.02	

^{a,b} - significantly different.

DISCUSSION

There are different factors which influences the maturation, fertilization and embryo development in vitro of animal oocytes. Rabbit oocytes were presently studied for the effect of LIF on maturation, fertilization and embryo development. The maturation and fertilization rates were increased significantly when LIF at a concentration of 10 ng/ mL is added in the respective medium. The mechanism of action of LIF has to analyzed. LIF exerts a number of different biological effects upon varied neuronal cells. Firstly, LIF has been observed to stimulate a switch in neurotransmitter

phenotype of parasympathetic nerves from adrenergic to cholinergic. These facts are to be studied for rabbit oocytes and embryos.

It is reported in sheep that LIF exerted a positive influence on the quality of the blastocysts as revealed by significantly higher number of ICM cells and total number of cells. (.Grazyna Ptak et al.,2006). in vitro blastocyst hatching rates were significantly improved in the presence of LIF ($P < 0.02$). Number of total cells and of inner cell mass (ICM) cells was increased in LIF treated bovine blastocysts (Sirisathien S, 2003). The total cell mass for rabbit blastocyst also increased when treated with 10 ng / mL of LIF. The effect on blastocyst development keeping in view of cellular differentiation has to study in detail for successful stem cell production in rabbit.

CONCLUSION

In conclusion LIF exerts influence on in vitro maturation, fertilization and blastocyst development on rabbit oocytes.

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