



Effect of Leukemia Inhibitor Factor (LIF) on *in vitro* maturation and fertilization of matured cattle oocytes

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Abstract

Effect of Leukemia Inhibitor factor (LIF) on *in vitro* maturation and fertilization of matured cattle oocytes were tested using frozen thawed semen. Oocytes collected from ovaries of slaughtered cattles were matured at *in vitro* conditions and fertilized with frozen thawed semen in the fertilization medium with different LIF concentrations. Maturation rate and Fertilization rate for five different bulls was determined. A maximum maturation rate was observed at a LIF concentration of 20 $\mu\text{g/mL}$ while the maximum fertilization rate obtained was also at a LIF concentration of 10 $\mu\text{g/mL}$.

Keywords: cattle, *in vitro* maturation, *in vitro* fertilization, LIF oocytes

Leukemia inhibitory factor (LIF) is a 45-56 kDa glycoprotein that has an important role in proliferation and embryo implantation. Its effect on oocyte maturation and how to exert the function remained to be elucidated. *In vitro* maturation and fertilization studies have been carried out successfully in several mammalian species like mouse, sheep, rabbit and buffalo¹⁻⁶. Different growth factors effect the *in vitro* maturation, fertilization and development. LIF is one of such factors that affect the maturation, fertilization rate and embryonic development and therefore, objective of the study was to establish the same and to optimize the concentration for obtaining a maximum *in vitro* maturation and fertilization rate for *in vitro* cattle oocytes⁶⁻¹⁰.

Media for *in vitro* maturation and fertilization were prepared following the methods of Totey *et al* and Ball *et al*^{4,5}, and filtered, sterilized and pre incubated at 38°C for 3 h prior to use. Oocytes were collected from ovaries of slaughtered cattle. Ovaries were transported from the slaughter-house in a thermos flask with normal saline at 32°C and with 2 mg/mL of gentamicin. Collection and maturation of oocytes was done as described earlier⁴. Frozen thawed semen collected from Kerala Livestock Department was used for the fertilization studies^{11,12}. One straw of semen of a particular bull was taken out. Dead and live sperms were separated by percoll method. It was then fertilized with *in vitro* matured oocytes. The effect of heparin on *in vitro* fertilization was tested by adding it at various concentrations (0, 1, 10, 100 µg/mL) to the fertilization medium^{13,14}. Oocytes were incubated for 16-18 h at 38°C in a CO₂ incubator and taken out, washed, fixed and stained with orcein and classified as fertilized and nonfertilized. Oocytes with clear cytoplasm and well developed cumulus cells were graded as good quality matured oocytes.

Fertilized oocytes were having two pronucleus⁴. The experiment was carried out for 100 numbers of good quality oocytes for each concentration of LIF for maturation. Then fertilization rate were also tested with LIF at different concentration for each bull, using good quality oocytes.. Similarly, *in vitro* fertilization for five bulls was conducted and their fertilization rates determined. The experiment was repeated thrice with different concentration of LIF. The number of oocytes taken for each trial varied from 98 to 108 since some of the oocytes underwent degeneration.

Results are summarized in Table 1 and Table 2

Table 1. Effect of LIF on *in vitro* maturation of cattle oocytes

Animal no.	LIF concentration ig/MI)	No of oocytes used for maturation	No of oocytes matured	% maturation *
1	0	105	25	22
	5	100	33	30
	10	108	59	58.5
	20	100	75	75.2
	100	100	35	32

* Total number of matured oocytes varies since some are degraded.

Table 2. Effect of LIF on in vitro fertilization of cattle oocytes

Animal no.	LIF concentration (g/mL)	No of oocytes taken for fertilization	No of oocytes fertilized	% fertilization obtained
1	0	105	8	7.2
	5	100	15	13.0
	10	108	43	42.1
	20	100	55	23.0
	100	100	23	15.0
2	0	100	10	9.0
	5	99	18	17.1
	10	103	26	25.2
	20	104	49	48.2
	100	102	37	37.0
	10			
3	0	101	7	6.7
	5	100	14	13.6
	10	100	26	25.1
	20	100	37	37.0
	100	105	23	22.8
4	0	98	12	12.4
	5	104	28	28.0
	10	99	45	44.2
	20	100	58	58.0
	100	103	34	35.0

Oocytes with LIF concentration of 20 $\mu\text{g/mL}$ showed a maximum maturation of 75.2%. When the concentration was increased to 30 $\mu\text{g/mL}$ the rate was decreased.

Maximum fertilization rate for each LIF concentration was calculated. It varied significantly for different concentration of LIF. It showed a significant increase in maximum fertilization rates of 58%, 48.2% and 42.% and 37% respectively at a heparin concentration of 10 $\mu\text{g/mL}$. However, the maximum rate of fertilization achieved at a LIF concentration of 20 $\mu\text{g/mL}$ were varied from animal to animal.

The results showed that the maximum normal fertilization rate obtained was significantly different from the fertilization rate obtained for each bull without LIF. This confirms the fact that the rate of fertilization depends on the composition of the maturation and fertilization medium^{9,14,15,16}

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References

- 1 Choi T S, Mori M, Kohmoto K and Shoda Y, 1987. Beneficial effect of serum on the fertilizability of mouse oocyte matured in vitro, *J Reprod Fertil*, **79**: 565-568.
- 2 Cheng W T K, Moor R M and Polge C, 1986. *In vitro* fertilization of pig and sheep oocytes matured *in vivo* and *in vitro*, *Theriogenology*, **25**: 25-46.
- 3 Chang M.C., 1959. Fertilization of rabbit ova *in vitro*, *Nature (Lond)*, **23**: 466-467.
- 4 Totey S M, Singh G and Talwar G P, 1992. *In vitro* maturation, fertilization and development of buffalo oocytes: Effect of media, hormone and sera, *Theriogenology*, **39**: 597-607.
- 5 Ball G B, Leibfried M L, Lenz R W, Ax R L, Bavister B B and First N L, 1983. Factors affecting successful IVF of bovine follicular oocytes, *Biol Reprod*, **28**: 717-725.
- 6 Parrish J J, Susko-Parrish J L and First N L, 1985. *In vitro* fertilization of bovine oocytes using heparin treated and swim up separated frozen spermatozoa produced repeatable and in high frequencies of fertilization, *Theriogenology*, **26**: 216 (Abstr).
- 7 Parrish J J, Susko-Parrish J L, Winer M A and First N L, 1988. Capacitation of bovine sperm by heparin, *Biol Reprod*, **38**: 1171-1180.
- 8 Kiang S, Yang Y, Chang S and Foote R, 1991. Effect of sperm capacitation and oocyte maturation procedures on fertilization and development of bovine oocytes *in vitro*, *Theriogenology*, **35**: 218-222.
- 9 Marks and Ax R L, 1986. Glycosaminoglycans in oviducts and their influence on acrosome reaction in bovine spermatozoa *in vitro*, *J Anim Sci*, 861-867.
- 10 Moor H D M and Bedford J M, 1978. An *in vivo* analysis of factors affecting the fertilization of hamster eggs, *Biol Reprod*, **19**: 879-885.
- 11 Fukui Y, Fukushima M and Ono H, 1983. Fertilization *in vitro* of bovine oocyte after different sperm procedures, *Theriogenology*, **20**: 651-660.
- 12 Niwa Kapok C K and Okuda K, 1991. Penetration *in vitro* of bovine oocytes during maturation of frozen thawed spermatozoa, *J Reprod Fertil*, **91**: 329-330.
- 13 Parrish J J, Susko-Parrish J L, Crister E S and First N L, 1986. Bovine *in vitro* fertilization with frozen thawed semen, *Theriogenology*, **25**: 591-600.

- 14 Parrish J J, Susko-Parrish J L and First N L, 1984. Effect of swim up separation and heparin treatment of frozen thawed spermatozoa on *in vitro* fertilization of bovine oocytes, *BiolReprod*, **30**: 112 (Abstr).
- 15 Parrish J J, Susko-Parrish J L and First N L, 1985. Role of heparin in bovine sperm capacitation, *Biol Reprod.*, **1**: 211
16. Raushedh *et al.*, 2010. Effects of Leukemia Inhibitory Factor on gp130 Expression and Rate of Metaphase II Development during *in vitro* Maturation of Mouse oocyte, *ran Biomed J.* Jul; **14**(3): 103–107