DonnishJournals

Donnish Journal of Medical Laboratory and Diagnosis
Vol 1(2) pp. 015-018 October, 2015.
http://www.donnishjournals.org/djmld

Original Research Article

Addition of Leukemia Inhibitory Factor (LIF) Could Benefit Clinical in Vitro Fertilization Culture

Amel Zhioua, Moez Kdous*, Nadia Mami, Khadija Kacem, Hanene Elloumi, Belkis Henidi, Mohamed Khrouf and Fethi Zhioua

Center of Reproductive Biology, Department of Gynecology and Obstetrics, Aziza Othmana Hospital, Tunis, University of Tunis El Manar, Tunisia.

Accepted 19th June, 2015.

Objective: **This study aimed to assess whether the addition of LIF (leukemia inhibitory factor) into a specific culture medium will increase the embryo quality susceptible to enhance pregnancy rate following embryo transfer.** ***Methods:*** **This is a blind prospective study, conducted on 2,192 oocytes received the day of ovum pick-up from 326 women undergoing the ICSI program. In each microdroplet of embryo culture medium containing an oocyte microinjected, LIF was randomly added on day 0 or on day 1 after the microinjection, and the microinjected oocytes were incubated individually. The embryo quality on day 2 and day 3 (blastomere number, blastomere regularity and fragmentation rate) was compared.** ***Results:*** **The number of four-cell embryos (on day 2), eight-cell embryos (on day 3) and embryos with regular blastomeres in both days were higher with LIF. The adjunction of LIF just after the microinjection provided better results than 24 hours after the microinjection. A statistically significant improvement was observed in embryos with a low fragmentation rate on both days 2 and 3. LIF also seems to improve pregnancy, implantation and live birth rates, but with no significant difference.** ***Conclusion:*** **Addition of LIF to embryo culture media after ICSI program has a beneficial effect on human embryos.**

Keywords: LIF, Embryo quality, Embryo culture media, ICSI.

INTRODUCTION

The early stages of embryonic development are regulated by an array of cytokines and growth factors which play a fundamental role in the synchronization of the development of the conceptus and the endometrium throughout the implantation period. The expression of most of these factors and their receptors occurs both in the embryo and in the woman's reproductive tract making an expression in mirror; but the mechanisms of action of these factors are not well known yet.

LIF is a member of interleukin-6 family and one of the most important cytokines essential for successful implantation. LIF is expressed in both the embryo and the endometrium. Mutation of the LIF gene in female mice prevents embryo implantation (Stewart and al., 1992; Cheng and al., 2002) and the uterus shows little evidence of decidualization (Chen and al., 2000). LIF has potential biological effects on several cell types in the early embryo and on the trophectoderm cells and it also influences the early events of implantation by acting on the luminal epithelium, glandular epithelium and even on the stroma (Fry, 1992).

Furthermore, a correlation has been suggested between LIF and LIF-receptors levels and pinopod formation (Agadjanova and al., 2003). In LIF-null animals, pinopods do not develop over the apical cell membranes, which remain microvillous (Fouladi-Nashta and al., 2005). The precise level needed for the effective action of LIF is controversial; first, the levels of LIF in uterine flushings were suggested to be lower in women with unexplained infertility compared with control fertile women (Laird and al., 1997); but a few years later, another study reported that lower levels of LIF are predictive of implantation success and higher levels are indicative of inflammation (Ledee-Bataille and al., 2002).

In this prospective randomized study, we have introduced LIF into a specific culture medium of microinjected oocytes to explore its possible role in early embryonic development by analysis of the embryo quality on day 2 and 3 after microinjection.

MATERIAL AND METHODS

This is a blind prospective study, conducted from March 2012 to February 2014, on 2,192 oocytes received the day of ovum pick-up from 326 women undergoing the ICSI program. A fully informed written consent was taken from all the patients. The

*Corresponding Author: Moez Kdous, Center of Reproductive Biology, Department of Gynecology and Obstetrics, Aziza Othmana Hospital, Tunis, Medicine School of Tunis, University of Tunis El Manar, Tunisia. Tel: + 00216 21852683 Fax: + 0021671260174. E-mail: kdousmoez@gmail.com

study protocol was in accordance with the guidelines of the Declaration of Helsinki and was approved by the Institutional Ethics Committee.

Criteria for inclusion in this study:

1- a normal ovarian function
2- a history of ≤ 3 attempts at IVF or ICSI
3- serum FSH on day 2 or 3 ≤ 15 IU/ml
4- estradiol ≤ 60 pg/ml
5- number of mature collected oocytes ≥ 6

The women's age ranges from 21 to 41 years.
The oocyte cohort of each woman was randomly divided into two groups:

- Group 1 (n = 1122): Oocytes exposed to LIF after microinjection.
- Group 2 (n = 1070): Oocytes not exposed to LIF after microinjection.
- *G*roup 1 (exposed to LIF) was composed of two sub-groups:
- Subgroup 1a (n = 748): Oocytes exposed to LIF, just after microinjection (LIF day 0).
- Subgroup 1b (n = 374): Oocytes exposed to LIF, 24 hours after microinjection (LIF day 1).

In each microdroplet of embryo culture medium G-1^{TM} Plus, Vitrolife (Sweden) containing an oocyte microinjected, lyophilized recombinant human LIF Invitrogen, GIBCO (USA) was added at a concentration of 2.5µg/ml. LIF was randomly introduced on day 0 or on day 1 after the microinjection, and the microinjected oocytes were incubated individually for 2 to 3 days.

For each group (exposed and non exposed embryos to LIF) and subgroup (exposed on day 0 and exposed at day 1), we compared the embryo quality on day 2 and day 3 (blastomere number, blastomere regularity and fragmentation rate). The biologist, who observed the embryonic grade, was blinded to oocytes groups.

STATISTICAL ANALYSIS

For both groups and subgroups, the results were expressed as frequencies. To make them comparable, these results were transformed into a logarithmic version then analyzed according to the method of ANOVA using SPSS 17.0 software. Statistical significance was assigned at $P < 0.05$.

RESULTS

The present study showed an improvement in embryo quality on day 2 and especially on day 3 after exposure to LIF during early stages of embryonic development. The use of LIF significantly increased the number of embryos with a low fragmentation rate on day 3 (60.4% vs 50.3%, $p<0.05$). It also improved the number of four-cell and eight-cell embryos on day 2 and 3, respectively, and the number of embryos with regular blastomeres on both days, but the difference was not significant (Table 1).

Group 1 (exposed to LIF) was composed of two sub-groups: Subgroup 1a: Oocytes exposed to LIF just after microinjection (LIF day 0) and Subgroup 1b: Oocytes exposed to LIF 24 hours after microinjection (LIF day 1). The adjunction of LIF just after the microinjection provided better results than 24 hours after the microinjection. Indeed, a statistically significant improvement was observed in embryos with a low fragmentation rate on day 2 (71.3% vs 49.6%, p=0.001) and also on day 3 (70.9% vs 31%, p=0.001).

In the subgroup LIF day 0, better results were observed on day 3 concerning the blastomere number and regularity, although this difference failed to reach statistical significance (Table 2). LIF also seems to improve pregnancy, implantation and live birth rates, but with no significant difference (Table 3).

DISCUSSION

Implantation is a very intricate process, which is controlled by a number of complex molecules like hormones, cytokines, and growth factors and their cross talk (Singh and al., 2011). They act and are produced on both sides of the fence (Bischof & Cohen, 2010); but their mechanisms of action are very complex and not completely understood at present.

LIF is one of the most important cytokines interfering with implantation. It is a polyfunctional highly glycosylated glycoprotein that occurs naturally in a range of molecular weights from 38 to 67 KDa, it influences a wide range of cell types in different ways. This pleiotropic cytokine can be produced in many or perhaps all body tissues (Metcalf, 2003). In the endometrium, its expression depends on the phase of the menstrual cycle, with a striking increase in the mid and late secretory phase coinciding with the window of implantation; so, LIF is the best example of how hormones act through cytokines. Thus, LIF has been proposed as a molecular marker of receptive endometrium. LIF mRNA and protein are localized in the luminal and glandular epithelium and in the stroma cells (Cullinan and al., 1996; Vogiagis and al., 1996)

As to current knowledge, LIF binds with LIF receptor (LIFR) of a plasma membrane with gp 130 glycoprotein (the common signaling receptor for IL-6 family cytokines) to form a high affinity receptor through which LIF signaling is triggered (Heinrich and al., 2003**).** The LIFR activates several signaling pathways in diverse cells types, including the Jak/STAT, MAP kinase (MAPK), and PI3-kinase (PIPK) pathways (Duval and al., 2000). LIFR is expressed both in the luminal epithelium of the endometrium and in the blastocyst. Thus, LIF may signal to both uterine and embryonic cells during implantation (Laird and al., 1997). In the mouse blastocyst, LIF is expressed by extraembryonic membranes (from early to fully expanded blastocoel cavity).

Most authors report that only at the blastocyst stage the embryo can express LIF and LIFR (Sun & Shi, 1998), and that LIF can improve embryo development in vitro only at this stage; so they consider that LIF can be beneficial exclusively for preimplantation embryos however it does not affect early embryonic development (Hsieh and al, 2000; Sargent and al, 1998). Authors indicate that there is no interest to supplement the culture media with LIF at the earlier stage of the embryo development (Lavranos and al, 1995).

The present study is the first one to demonstrate that the exposure to LIF during early stages of embryonic development shows an improvement in embryo quality especially on day 3. LIF increased the number of embryos with low fragmentation rate, with a statistically significant difference on day 3. The number of four-cell embryos (on day 2), eight-cell embryos (on day 3) and embryos with regular blastomeres on both days were also higher with LIF.

This action can be explained by the possibility of direct effects of this cytokine on properties of biological membranes.

Table 1: Embryo quality in the 2 groups: (LIF+)/(LIF-)

	EMBRYO QUALITY	GROUP 1 (LIF+) (n=1122)	GROUP 2 (LIF -) (n=1070)	P value
Day 2	4-Cell embryos	47.7%	46.8%	NS
	Regular blastomeres	73.2%	68.6%	NS
	Low fragmentation rate	63.5%	58.9%	NS
Day 3	8-Cell embryos	20.5%	17.3%	NS
	Regular blastomeres	71.3%	62.7%	0.069
	Low fragmentation rate	**60.4%**	**50.3%**	**0.047**

NS: not significant

Table 2: Embryo quality in the 2 subgroups: (LIF day 0)/(LIF day 1)

	EMBRYO QUALITY	SUBGROUP 1a (LIF day 0) (n=748)	SUBGROUP 1b (LIF day 1) (n=374)	P value
Day 2	**4-Cell embryos**	46.8%	59.3%	NS
	Regular blastomeres	74.1%	71.7%	NS
	Low fragmentation rate	**71.3%**	**49.6%**	**0.001**
Day 3	**8-Cell embryos**	23.1%	13.6%	NS
	Regular blastomeres	73.5%	65.1%	NS
	Low fragmentation rate	**70.9%**	**31%**	**0.001**

NS: not significant

Table 3: Pregnancy outcomes of the three groups of patients LIF+ embryos, LIF- embryos and LIF+ embryos with LIF- embryos.

	GroupA (LIF+) (n=140)	GroupeB (LIF-) (n=136)	Groupe C (LIF+/LIF-) (n=50)	P value
Age range (years; mean ± SD)	32,1±3,2	33,2±4,5	31,4±3,7	NS
Clinical pregnancies (%)	38,5 (54/140)	29,4 (40/136)	32 (16/50)	NS
Implantations (%)	20,4 (58/284)	16 (44/274)	21,2 (20/94)	NS
Live births (%)	32,8 (46/140)	23,5 (32/136)	28 (14/50)	NS

NS: not significant

As a matter of fact, LIF is known to have three isoforms, one of which (tLIF) does not require a membrane receptor to act on the target cell (Mezhevikina and al., 2011). The recombinant LIF acts in the same way. The electrophysiological studies of Mezhevikina and al., 2011) demonstrated that recombinant LIF acts as a membranotropic agent: it affects the viscosity of membrane lipids (lipid bilayer), increases the positive charge and conductivity of cell membranes. This change of the cytoplasm membrane structure requires signals into cell susceptible to cause functional changes (Mezhevikina and al., 2011). Another main result of our study is that the action of LIF seems better if it is introduced precociously in the embryo culture medium.

The addiction of LIF just after the microinjection provided better results than 24 hours after the microinjection. Indeed, if the oocytes were in contact with LIF on day 0 just after microinjection a statistically significant improvement was observed in embryos with a low fragmentation rate; the number and regularity of blastomeres were also improved, although this difference failed to reach statistical significance. LIF also

seems to improve pregnancy, implantation and live birth rates, but with no significant difference. The absence of significance can be explained by the smaller number of patients. Group A is composed of patients who had had a homogenous transfer (LIF+ embryos); Group B (LIF- embryos) and group C (LIF+ and LIF- embryos) (Table 3).

In conclusion, LIF was demonstrated to be able to improve the embryo quality, thus it can be interesting in embryonic culture media as a medium supplement. The use of LIF in order to improve the rates of pregnancy and live birth should be further investigated in ICSI cycles.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. Agadjanova, L., Stavreus-Evers, A. & Nikas, Y. (2003). Coexpression of pinopodes and leukemia inhibitory factor, as well as its receptor, in human endometrium. Fertility and Sterility, 79, 808-814.
2. Bischof, P. & Cohen, M. (2010). Endometrial factors and blastocyst implantation. Indian Journal of Physiology and Pharmacology, 54(5), 7-16.
3. Chen, J.R., Cheng, J.G., Shatzer, T., Sewell, L., Hernandez, L. & Stewart, C.L.(2000). Leukemia inhibitory factor can substitute for nidatory estrogen and is essential to inducing a receptive uterus for implantation but is not essential for subsequent embryogenesis. Reproduction-Development, 141(12), 4365
4. Cheng, J.G., Rodriguez, C.I. & Stewart, C.L. (2002). Control of uterine receptivity and embryo implantation by steroid hormone regulation of LIF production and LIF receptor activity: towards a molecular understanding of 'the window of implantation'. Reviews in Endocrine and Metabolic Disorders, 3, 119–126.
5. Cullinan, E.B., Abbondanzo, S. J., Anderson, P. S., Pollard, J.W., Lessey, B. A. & Stewart, C. L. (1996). Leukemia inhibitory factor (LIF) and LIF receptor in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. Proceedings of the national academy of sciences of the United States of America, 93, 3115-3120.
6. Duval, D., Reinhardt, B., Kedinger, C. & Boeuf, H. (2000). Role of suppressors of cytokine signaling (Socs) in leukemia inhibitory factor (LIF) –dependent embryonic stem cell survival. The FASEB Journal, 14, 1577–1584.
7. Fouladi-Nashta, A.A., Jones, C.J., Nijjar, N., Mohamet, L., Smith, A., Chambers, I. & Kimber, S.J. (2005). Characterization of the uterine phenotype during the peri-implantation period for LIF-null, MF1 strain mice. Developmental Biology, 281, 1–21.
8. Fry, R.C. (1992). The effect of leukemia inhibitory factor (LIF) on embryogenesis. Reproduction, Fertility and Development, 4, 449-458
9. Heinrich, P.C., Behrmann, I., Haan, S., Hermanns, H.M., Muller-Newen, G. & Schaper, F. (2003). Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochemical Journal, 374, 1-20.
10. Hsieh, Y.Y., Tsai, H.D., Chang, C.C., Hsu, L.W., Chang, S.C. & Lo, H.Y. (2000). Prolonged culture of human cryopreserved embryos with recombinant human leukemia inhibitory factor. Journal of Assisted Reproduction and Genetics, 17, 131-134.
11. Laird, S.M., Tuckerman, E.M., Dalton, C.F., Dunphy, B.C., Li, T.C. & Zhang, X. (1997). The production of leukaemia inhibitory factor by human endometrium: presence in uterine flushings and production by cells in culture. Human Reproduction, 12, 569–574
12. Lavranos, T.C., Rathjen, P.D. & Seamark, R.F. (1995). Trophic effects of myeloid leukaemia inhibitory factor (LIF) on mouse embryos. Journal of reproduction and fertility, 105, 331-338.
13. Ledee-Bataille, N., Lapree-Delage, G., Taupin, J.L., Dubanchet, S., Frydman, R. & Chaouat, G. (2002). Concentration of leukaemia inhibitory factor (LIF) in uterine flushing fluid is highly predictive of embryo implantation. Human Reproduction, 17, 213–218
14. Metcalf, D. (2003). The unsolved enigmas of leukemia inhibitory factor. Stem Cells, 21, 5-14.
15. Mezhevikina, L.M., Petrova, R.R., Borisova, M.P., Seraya, O.L., Fesenko, E.E. (2011). Effects of Recombinant Leukemia Inhibitory Factor (LIF) on Functional Status of Mouse Embryonic Stem Cells. In Atwood, C. (Ed.), Embryonic Stem Cells: The Hormonal Regulation of Pluripotency and Embryogenesis (pp 79-90) ISBN: 978-953-307-196-1
16. Sargent, I.L., Martin, K.L. & Barlow, D.H. (1998). The use of recombinant growth factors to promote human embryo development in serum-free medium. Human Reproduction, 13, 239-248.
17. Singh, M., Chaudhry, P. & Asselin, E. (2011). Bridging endometrial receptivity and implantation: network of hormones, cytokines, and growth factors. Journal of Endocrinology, 210, 5–14.
18. Stewart, C.L., Kaspar, P. & Brunet, L.J. (1992) Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature, 359, 76–79.
19. Sun, H. & Shi, W.K. (1998). Expression of LIF gene during early development of mouse embryo. Shi Yan Sheng Wu Xue Bao, 31(1),105-109
20. Vogiagis, D., Marsh, M.M., Fry, R.C. & Salamonsen, L.A. (1996). Leukemia inhibitory factor in human endometrium throughout the menstrual cycle. Journal of Endocrinology, 148, 95-102