The Effect of Serum Concentration of Leukaemia Inhibitory Factor on *In Vitro* Fertilization Treatment Outcome

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Problem
To evaluate the association of peripheral leukaemia inhibitory factor (LIF) levels on implantation and miscarriage rates after *in vitro* fertilization (IVF) treatment.

Methods
Prospective observational study of 120 randomly selected women who underwent IVF treatment. The concentration of LIF in serum was determined by enzyme-linked immunosorbent assay.

Results
There was no significant differences with regard to the systemic mean LIF concentration between the pregnant (42 patients, LIF: 11.55 pg/mL ± 5.3 S.D.) and non-pregnant (66 patients, LIF: 13.47 pg/mL ± 5.1 S.D.) women after IVF treatment. Likewise, for those women who have positive pregnancy after IVF treatment, the systemic mean LIF levels were not significantly different between women who have an ongoing pregnancy (34 ongoing pregnancy, LIF: 11.26 pg/mL ± 5.2 S.D.) and those who had miscarriage (eight miscarriage, LIF: 12.78 pg/mL ± 5.6 S.D.).

Conclusion
The systemic levels of LIF concentration have no association with implantation rate or miscarriage rate in women undergoing IVF treatment. Measuring serum LIF concentration prior to embryo transfer in IVF treatment has no predictive value of implantation rate or miscarriage rate.

Introduction

Leukaemia inhibitor factor (LIF) is a pleiotropic cytokine that belongs to interleukin (IL)-6 family. It has the ability to inhibit proliferation of the myeloid leukaemic cell line and it also regulates the growth and differentiation of embryonic stem cells, primordial germ cells and endothelial cells. LIF is known to be produced by lymphocytes, monocytes, macrophages and some epithelial cells. Stewart et al.[1] reported that blastocyst implantation is influenced by maternal expression of LIF and it has been suggested that human oocytes and pre-implantation embryos express LIF receptors and have a high affinity to maternal LIF.[2,3] Furthermore, Kojima et al.[4] reported that there are high expressions of LIF in human endometrium and placenta. Dunglison et al.[5] demonstrated that LIF significantly enhances the blastocyst formation rate of human embryos cultured in serum free medium and improves embryo quality. LIF expression in the endometrium increases around the time of blastocyst implantation and endometrial biopsies from women with proven fertility reveal LIF mRNA expression during the secretory phase with a peak between days 18 and 25 of menstrual cycle.[6,7] All these studies suggested that there is a strong evidence that LIF plays an important role in implantation of human embryos. In addition, some previous studies reveal that the LIF concentration in uterine flushings from women with unexplained infertility was found to be significantly lower during the luteal phase of the menstrual cycle than in women with proven fertility.[8,9] Hambartsoumian[10] reported that LIF production in cultured endometrial tissue from women with unexplained infertility and women with repeated implantation failure after IVF treatment was markedly reduced in comparison with fertile women. Arici et al.[11] have reported LIF expression in human follicular fluid and ovarian cells. To our knowledge, there are no previous studies investigating the relationship between systemic levels of LIF and *in vitro* fertilization (IVF) treatment outcome. It has been reported that systemic administration of LIF can improve implantation after IVF treatment with the speculation that systemic levels of LIF will have an imperative effect on uterine LIF levels and embryo implantation.[12] The aim of this study was to evaluate the relationship between the peripheral LIF level and the implantation rate, and miscarriage rate after embryo transfer (ET) in women having IVF treatment.

Materials and methods

Study Population

One hundred and twenty patients undergoing IVF-ET treatment cycles were recruited into the study. Independent ethical approval was obtained from the Local Research Ethics Committee. Exclusion criteria: women with known immunological disease (antiphospholipid antibodies, lupus anticoagulant, anticardiolipin antibodies), uterine abnormality (fibroid, uterine polyp, uterine septum), less than two embryos available for transfer or endometrium thickness <8 mm before ET. Blood samples were obtained on the day of vaginal egg collection prior to the procedure. Informed consent was provided by all subjects at recruitment.

Stimulation Protocol

Pituitary down regulation was achieved with either Nafarelin or Buserelin at mid luteal phase. Ovarian stimulation was carried out with either recombinant follicle-stimulating hormone (FSH), human menopausal gonadotrophin or urinary FSH. When follicles reached pre-ovulatory size (18–22 mm), 10,000 IU of human chorionic gonadotrophin (hCG) was administered. Oocytes were aspirated using trans-vaginal ultrasound guidance 34–36 hr after hCG administration. All embryos were allowed to cleave and the best two or three embryos were selected for transfer. Embryo transfer was performed on day 2 or day 3 using a soft catheter (Wallace, Kent, UK) with trans-abdominal ultrasound guidance. Progesterone supplement for luteal support (Cyclogest; Shire Pharmaceuticals Ltd, Hants, UK), 400 mg once a day per-vaginum or per-rectum, was commenced 1 day before ET and continued until a pregnancy test was performed 2 weeks after ET.

Human LIF Cytokine Assay

A commercial (Bender Medsystems, Burlingame, CA, USA) enzyme-linked immunosorbent assay (ELISA) was used for measuring LIF concentration. Plates were coated with anti-LIF antibody at a concentration of 2.5 μg/mL in phosphate-buffered saline (PBS) and incubated overnight. Assay buffer (PBS, 0.5% bovine serum albumin, 0.05% Tween-20) was added to the wells (acting as a blocker) and plates were washed in PBS + 0.05% Tween-20. Samples, blank and standards were added to the appropriate wells. After incubation plates were washed and a secondary anti-LIF monoclonal antibody biotin-labelled conjugate was added. Plates were incubated and washed. Streptavidin-Horseradish peroxidase (HRP) was added, plates were washed and a Tetra-methyl Benzidene (TMB) substrate was added. A solution of 4 N sulphuric acid was used to stop the reaction. Plates were read in an automatic plate reader at a wavelength of 450 nm with a reference wavelength of 620 nm. The range of detection of the assay is from 3.13 to 200 pg/mL. The concentrations of LIF in the samples were calculated from the standard curve created from controls.

Data Analysis

All IVF-ET data were collected in Medical System for IVF (MedicalSys, London, UK) and analysed by

Statistics Package for Social Sciences (SPSS, Surrey, UK). Descriptive statistical analysis was performed initially to examine the normal distribution of all continuous variances for parametric statistical tests. Analysis of variance was then conducted to assess the duration and amount of gonadotrophin required to achieve follicular maturity, oestradiol levels on hCG day, number of mature follicles, number of available embryos for transfer, number of oocytes collected, fertilization rate and LIF levels between the pregnant and non-pregnant groups.

Results

Of the 120 women who underwent IVF-ET, 12 were excluded from statistical analysis. Of these 12, four had failed fertilization, four had only one embryo available for transfer, one had ovarian hyperstimulation syndrome and therefore did not have ET, two had an endometrial thickness <7.5 mm and one woman had poor quality embryos. None of the women who participated in the study were excluded because of abnormal uterine anatomy or known previous abnormal immunological tests.

Table I shows patient treatment outcome, LIF levels, mean age, duration of infertility, basal FSH levels, mean number of previous IVF attempts, number of previous miscarriages and outcome of ovarian stimulation in pregnant and non-pregnant group. There were no significant differences between the two groups with regard to all the parameters.

Table II examines the relationship between LIF concentration levels, mean age, duration of infertility, basal FSH levels, mean number of previous IVF attempts, number of previous miscarriages and outcome of ovarian stimulation with pregnancy outcome in women with an ongoing pregnancy and those who miscarried. There were no significant differences between the two groups with regard to all the parameters. The LIF concentration was higher in the ongoing pregnancy group but statistically not significant.

Discussion

Failure of implantation after ET in IVF treatment is the major factor restricting the success of Artificial reproductive technique (ART). Only 20–30% of the embryos transferred after IVF treatment are able to implant and achieve a pregnancy.[13,14] These failures of implantation could be due to embryo factors, i.e. chromosomal abnormality of the embryos[15] or inadequate endometrial receptivity or defects in the embryo–endometrium interaction.[16,17] There is still no complete understanding of the molecular aspects of implantation, it does however involve a group of autocrine/paracrine molecules including cytokines, growth factors, adhesion molecules and invasive proteinases. Of all the cytokines identified in the endometrium LIF appears to be one of the most important mediators.

During placentation, the uterus responds to an implanting blastocyst by undergoing extensive modifications that result in transformation of the endometrium into a deciduas.[18] Stewart[19] reported that, in an animal LIF gene knock out model, unsuccessful implantation was exhibited by LIF−/− females

Table I Ovarian stimulation outcome and LIF concentration between pregnant and not pregnant women after IVF treatment

	Non-pregnant	Pregnant	*P*-value
Number of patients	66	42	NA
Leukaemia inhibitory factor concentration (pg/mL) ± S.D.	13.47 ± 5.1	11.55 ± 5.3	NS
Mean age ± S.D.	35.5 ± 3.7	34.1 ± 3.8	NS
Duration of infertility (years ± S.D.)	4.52 ± 2.7	3.36 ± 2.2	NS
Basal FSH levels (IU/L)	8.37	7.47	NS
Mean number of previous failed *in vitro* fertilization attempts	1.83	1.38	NS
Number of previous miscarriage	0.23	0.31	NS
Gonadotrophin[a] (IU)	3116.4	2651.7	NS
Average no. of oocytes collected ± S.D.	12.1 ± 5.8	12.7 ± 6.7	NS
Fertilization rate (%)	66.9	64.1	NS
Average no. of available embryos for transfer ± S.D.	7.66 ± 4.6	8.62 ± 5.5	NS
Average no. of embryos transferred	2.14	2.05	NS

NS, difference not statistically significant (*P* > 0.05); NA, not applicable; FSH, follicle-stimulating hormone.
[a]Mean amount of gonadotrophin used for stimulation in IU (recombinant FSH, human menopausal gonadotrophin or urinary FSH).

Table II Ovarian stimulation outcome and LIF concentration between women with ongoing pregnancy and miscarriage after IVF treatment

	Ongoing pregnancy	Miscarriage	*P*-value
Number of patients	34	8	NA
Leukaemia inhibitory factor concentration (pg/mL) ± S.D.	11.26 ± 5.2	12.78 ± 5.6	NS
Mean age ± S.D.	34.1 ± 3.7	34.5 ± 4.3	NS
Duration of infertility (years ± S.D)	3.17 ± 2.1	3.63 ± 2.9	NS
Basal FSH levels (IU/L)	6.76	8.26	NS
Mean number of previous failed *in vitro* fertilization attempts	1.13	2.88	NS
Number of previous miscarriage	0.22	0.38	NS
Gonadotrophin[a] (IU)	2528.2	2746.8	NS
Average no. of oocytes collected ± S.D.	12.2 ± 6.5	12.8 ± 8.1	NS
Fertilization rate (%)	71.2	69.4	NS
Average no. of available embryos for transfer ± S.D.	8.8 ± 5.1	9.5 ± 7.1	NS
Average no. of embryos transferred	2.04	2.00	NS

NS, difference not statistically significant ($P > 0.05$); NA, not applicable; FSH, follicle-stimulating hormone.
[a]Mean amount of gonadotrophin used for stimulation in IU (recombinant FSH, human menopausal gonadotrophin or urinary FSH).

and this may be a result of impaired uterine ability to undergo decidualization. In this model, implantation and decidualization were restored by administration of exogenous LIF. In an *in vitro* human tissue model, Sawai et al.[20] revealed that LIF plays a putative role in decidual cell function and formation. Therefore, all these studies suggest that LIF plays a vital role in embryo implantation. However, all these aforementioned studies are either animal or *in vitro* models, which may not accurately reflect human *in vivo* conditions.

Laird et al.[8] reported that LIF concentrations in uterine flushings from women with unexplained infertility were significantly lower than those from normal fertile women, and they suggested that LIF plays an important role in human embryo implantation. In contrast, Ledee-Bataille et al.[21] reported that a high concentration of LIF in uterine flushings was associated with poorer embryo implantation in women undergoing IVF treatment, and furthermore, from the same research group, Olivennes et al.[22] reported that the LIF concentrations in uterine flushings have no association with IVF treatment outcome. Therefore, there is still no consensus of opinion about how LIF concentrations in the uterine cavity affect embryo implantation.

Our study shows that there is no association between systemic concentration of LIF and treatment outcome, i.e. pregnant or non-pregnant, and pregnancy outcome, i.e. ongoing pregnancy or miscarriage, in women undergoing IVF treatment. This finding suggests that systemic levels of LIF may not have any effect on embryo implantation and development of pregnancy. Therefore, if there is any, the effect of LIF on embryo implantation and the development of pregnancy is most likely to be a localized effect rather than from a systemic influence. Some researchers in the field have suggested that i.v. infusion of recombinant LIF may improve implantation rate in IVF treatment[23] and Brinsden et al.[12] have reported that i.v. infusion of recombinant human LIF might benefit women with a history of recurrent failed implantation to improve implantation rate in IVF treatment in a proof of concept study. Those suggestions are partially in contrast with our finding because the results of our study show that there is no association between systemic concentrations of LIF with implantation rate and miscarriage rate. Therefore we speculate that the effect, if there is one, is a localized rather than systemic, and i.v. administration of LIF will have no significant impact on embryo implantation. Brinsden et al.[12] study used a highly selective study group with a small sample size. In the study by Brinsden et al.,[12] 52 women having IVF treatment were recruited and randomized into i.v. infusion of placebo or LIF treatment groups. The pregnancy rate was not significantly different between the placebo group (20%) and the LIF group (28%). In a subgroup analysis of women who had infertility not due to male factor, the pregnancy rate was significantly higher in the LIF group (28%, $n = 29$) when compared with the placebo group (0%, $n = 8$). A conclusion was made that i.v. infusion of recombinant human LIF can improve implantation rate in IVF treatment. With the small number of subjects in the subgroup analysis, the power of the study might not be statistically valid and hence the conclusion derived from this study may not be statistically significant, therefore a larger randomized study should be conducted to confirm this conclusion.

Conclusion

The systemic levels of LIF concentration have no association with implantation rate or miscarriage rate in women undergoing IVF treatment. This is, to our knowledge, the first study ever to evaluate the relationship between peripheral blood LIF levels and IVF treatment outcome. This is an important part of jigsaw, which can add to knowledge of the relationship between cytokines and implantation.

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